A SIMPLE COLORIMETRIC METHOD FOR THE ESTIMATION OF RELATIVE NUMBERS OF LACTOBACILLI IN THE SALIVA[1]

MARSHALL L. SNYDER

Hygienic Laboratory, University of Michigan, Ann Arbor, Michigan

This paper includes the results of an attempt to develop a simple colorimetric method for the estimation of the relative numbers of lactobacilli in the saliva as a supplementary procedure for the quantitative techniques (1, 2) used in the study of dental caries. The test was based on the assumption that since caries activity is closely correlated with the numerical rise and fall of lactobacilli in the saliva, it should be possible to demonstrate a corresponding increase and decrease in the amount of acid produced in a selective carbohydrate medium inoculated with definite amounts of saliva. Thus, significant color changes in selected time intervals could be interpreted roughly in terms of lactobacilli per c.c. of saliva as established by an adequate number of control plate counts. The present data embodies a large series of tests on specimens of saliva sent to the School of Dentistry of the University of Michigan for estimation of numbers of lactobacilli.

METHOD AND MATERIAL

The essential requirement for such a method was a medium selective for the acidogenic organisms, especially the lactobacilli, in the saliva and so colored that shifts in the indicator could easily be detected. Beef infusion agar to which glucose or lactose and an indicator were added was found to be very satisfactory since acid tomato-peptone agar (2) masked the color of the indicators used. Since the medium to be sufficiently selective must have an initial acidity of at least pH 5.0, the number of indicators was at once limited. After many trials with brom-cresol-green (pH 3.8–5.4), brom-phenol-blue (pH 3.0–4.6), benzene-azo-a-naphthylamine (pH 3.7–5.0), and a-naphthylamine-azo-sulfanilic acid (pH 3.5–5.7), it was found that brom-cresol-green was the most satisfactory because the color changes were easy to follow and the indicator was stable at sterilizing temperatures.

Using specimens of saliva from 38 selected caries-free children and 134 freshman medical students, it was found that "shake" tubes, to which measured amounts of saliva could be

[1] Supported in part by a grant from the Horace E. Rackham School for Graduate Study.

added at 45°C followed by immediate cooling of the medium and daily observation for 4 days at 37°C, gave more consistent results than either streaked plates or stabbed slants. These preliminary tests established a definite correlation between intensity and time of color change and estimated numbers of lactobacilli in the respective specimens.

Changes in the color of the indicator were recorded as 0 for no difference compared with uninoculated controls and 4 for a complete shift from the blue-green to the yellow. Intermediate stages were estimated as 1, 2 or 3 (*fig.* 1). However, to avoid the confusion in estimating degrees of intensity, significant or positive changes were considered to be only those tubes in which green was no longer the dominant color and determined as either 3 or 4. This point on the colorimetric scale for brom-cresol-green is approximately pH 4.2–4.4, and was selected because pure cultures of streptococci, yeasts, and staphylococci did not alter the color below this limit whereas cultures of lactobacilli, especially the S form, caused a complete shift in 48 hours.

To supplement the preliminary observations a survey by clinical and quantitative methods of the incidence of caries in a large number of children in a "mottled enamel" district in Texas[2] was welcomed. The specimens of saliva were collected in sterile bottles at different public schools and shipped by air mail to Ann Arbor where definite quantities were sampled before "shake" tube cultures were made. In this manner lactobacillus counts[3] were determined independently and were not compared with the results of the color tests until after the latter were discarded. All the results reported in this paper were determined with "shake" tubes which contained approximately 5–7 c.c. of the acid glucose or lactose agar containing brom-cresol-green as the indicator and inoculated with either 0.1 c.c. or 0.2 c.c. of saliva.

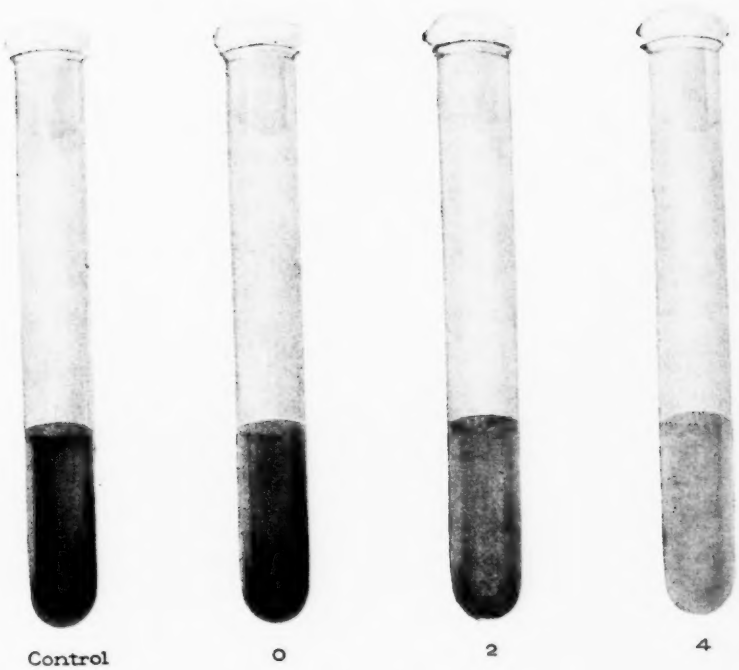
RESULTS

The significant color changes every 24 hours for 4 days with percentages of positive results in relation to independent lactobacillus counts are recorded in Tables I, II and III. Table I contains the data when 0.1 c.c. was inoculated into 2 per cent glucose infusion agar (pH 5.0) with bromcresol-green as the indicator. The lactobacillus counts were arbitrarily grouped.

Table I shows that in 24 hours only 1 specimen of saliva induced significant color change but in 48 hours the number of cultures showing distinct acid production increased proportionately with the estimated numbers of lactobacilli. At this time, however, only 2 of 158 specimens containing less than 1,000 lactobacilli per c.c. of saliva were recorded as positive. The same trend was observed at 72 and 96 hours with less clear cut features except the almost uniformly complete color shifts with lactobacillus counts above 20,000 per c.c. of

[2] Conducted for the U. S. Public Health Service by Dr. H. T. Dean and associates. Dr. Philip Jay was a consultant in this study.

[3] The author is indebted to Dr. Philip Jay of the School of Dentistry for the quantitative lactobacillus counts.



Changes in brom-cresol-green glucose agar (pH 5.0) in 48 hours inoculated with 0.2 c.c. saliva.

saliva. The failure to interpret color changes at 48 hours with lactobacillus counts between low and high numerical levels emphasized that acid production was not always dependent on numbers of lactobacilli and was correspondingly influenced by many factors, some of which will be mentioned later in this paper.

Similar tests were carried out with an increase of the inoculum to 0.2 c.c. of saliva. The results are given in Table II.

TABLE I

Comparison of acid tomato-peptone agar plate counts of lactobacilli in the saliva with color changes in brom-cresol-green glucose agar (pH 5.0) "shake" tubes inoculated with 0.1 c.c. saliva

LACTOBACILLI/C.C. SALIVA (TOMATO AGAR PLATE COUNTS)	NO. SPECIMENS	TIME IN HOURS							
		24		48		72		96	
		No. Positive*	Per Cent	No. Positive	Per Cent	No. Positive	Per Cent	No. Positive	Per Cent
0	114	0	0	1	0.88	3	2.6	8	7.0
0– 100	14	0	0	0	0	3	21.4	4	28.6
100– 1,000	30	0	0	1	3.33	19	63.4	26	86.7
1,000– 5,000	44	0	0	18	40.8	39	88.7	43	97.8
5,000– 10,000	25	0	0	14	56.0	23	92.0	24	96.0
10,000– 20,000	22	0	0	10	45.5	17	77.3	19	86.5
20,000– 30,000	19	0	0	14	73.7	18	94.7	19	100
30,000– 50,000	18	0	0	11	61.2	18	100	18	100
50,000–100,000	32	0	0	30	93.7	30	93.7	32	100
100,000–	45	1	2.2	40	89.0	42	93.5	43	95.7
	363								

* Positive changes included only those tubes in which green was no longer the dominant color, and recorded as 3 or 4.

In this series the same failure to produce significant amounts of acid in 24 hours was noted, but within 48 hours the number of cultures showing distinct color change was greatly increased. However, less than 2 per cent of the specimens having under 100 lactobacilli per c.c. of saliva were recorded as positive. Nearly uniform complete changes were observed with specimens containing over 100 lactobacilli per c.c. of saliva in 72 and 96 hours. It was apparent that increasing the inoculum from 0.1 c.c. to 0.2 c.c. of saliva caused a larger number of positive reactions in 48 hours with respect to lactobacillus popula-

TABLE II

Comparison of acid tomato-peptone agar plate counts of lactobacilli in the saliva with color changes in brom-cresol-green glucose agar (pH 5.0) "shake" tubes inoculated with 0.2 c.c. saliva

LACTOBACILLI/C.C. SALIVA (TOMATO AGAR PLATE COUNTS)	NO. SPECIMENS	TIME IN HOURS							
		24		48		72		96	
		No. Positive*	Per Cent	No. Positive	Per Cent	No. Positive	Per Cent	No. Positive	Per Cent
0	102	0	0	2	2.0	4	4.0	11	11.0
0– 100	18	0	0	0	0	6	33.3	12	67.0
100– 1,000	28	0	0	7	25.0	24	85.8	27	96.5
1,000– 5,000	50	0	0	21	42.0	42	84.0	47	94.0
5,000– 10,000	15	0	0	9	60.0	14	93.5	15	100
10,000– 20,000	18	0	0	12	67.0	17.5	94.5	18	100
20,000– 50,000	25	0	0	20	80.0	25	100	25	100
50,000–100,000	24	1	4.2	22	91.7	24	100	24	100
	280								

* Positive changes included only those tubes in which green was no longer the dominant color, and recorded as 3 or 4.

TABLE III

Comparison of acid tomato-peptone agar plate counts of lactobacilli in the saliva with color changes in brom-cresol-green lactose agar (pH 5.0) "shake" tubes inoculated with 0.2 c.c. saliva

LACTOBACILLI/C.C. SALIVA (TOMATO AGAR PLATE COUNTS)	NO. SPECIMENS	TIME IN HOURS							
		24		48		72		96	
		No. Positive*	Per Cent	No. Positive	Per Cent	No. Positive	Per Cent	No. Positive	Per Cent
0	29	0	0	1	3.5	2	6.9	2	6.9
0– 100	11	0	0	1	9.1	2	18.2	3	27.3
100– 1,000	22	0	0	2	9.1	14	63.7	19	86.5
1,000– 5,000	15	0	0	1	6.7	12	80.0	14	93.5
5,000– 10,000	7	0	0	3	43.0	7	100	7	100
10,000– 50,000	16	0	0	13	81.5	16	100	16	100
50,000–100,000	16	0	0	11	68.8	16	100	16	100
100,000–	26	0	0	21	80.8	26	100	26	100
	162								

* Positive changes included only those tubes in which green was no longer the dominant color, and recorded as 3 or 4.

tion. A few tests with 0.3 c.c. and 0.5 c.c. of saliva did not materially change the percentages already listed. As in the previous series there was the same objection of attributing acid production in 48 hours solely to numbers of lactobacilli, although the difference approached a minimum with a longer incubation period.

It was thought that another carbohydrate might give better differentiation; consequently, lactose was substituted for glucose in the acid infusion agar. This medium was inoculated with 0.2 c.c. of the respective specimens of saliva; the results are given in Table III.

Although this series was much smaller than the others, the relation of color changes to respective lactobacillus counts seemed to have much wider numerical limits; that is, of specimens with counts under 5,000 lactobacilli per c.c. of saliva only 6.5 per cent were positive, whereas all specimens of saliva with more than 5,000 lactobacilli per c.c. were strongly positive in 72 hours.

DISCUSSION

Since it has been established that caries remains absent or inactive with few or no lactobacilli in the saliva and becomes active when these forms appear in constant or increasing numbers, it is possible to predict caries activity by quantitative estimation of the numbers of lactobacilli in the saliva (3). At present, methods for estimating their numbers are limited to the laboratory; consequently it was attempted to develop for the practicing dentist a simple colorimetric test which would give essentially the same information easily and rapidly.

It was recognized that acid glucose broth cultures of saliva, although furnishing a valuable aid in detecting the presence of lactobacilli, gave no clue to the original numbers inoculated. In the same manner, final pH values of these broth cultures, as shown by Boyd, Zentmire and Drain (4), would in no way reflect the numbers in the inoculum. However, the use of a selective acid agar medium containing an indicator in "shake" tubes indicated in preliminary tests a correlation between color changes in this medium after inoculation with measured amounts of saliva and the estimated numbers of lactobacilli in the respective specimens. This relationship was partially confirmed in a large series of color tests controlled by separate lactobacillus counts. Thus, it was possible to select by negative color change

in 48 hours those specimens containing less than 1000 lactobacilli per c.c. of saliva with about 4 per cent error, but with increasing numbers of lactobacilli it was difficult to interpret color changes with numbers of lactobacilli until high numerical levels were reached where only a small percentage of specimens of saliva failed to induce color change in 48 hours. The failure to secure consistently positive results even with large numbers of lactobacilli in the saliva made clear the difference between what was being sought and actually what was happening; namely, the color changes reflected the total acid produced by acidogenic organisms in the saliva rather than numbers of lactobacilli. Any attempt, therefore, to correlate or interpret these findings in terms of caries or numbers of lactobacilli must include a consideration of many factors some of which may be briefly discussed.

In contrast to the quantitative method which gives relative but constant results for numbers of lactobacilli, despite all the errors that influence surface plate counts, the test tube procedure is one in which all the organisms of the saliva are incorporated into a selective but different and partially anaerobic medium. Although pure cultures of yeasts and streptococci did not cause significant changes, their influence on acid production in symbiosis with the lactobacilli must be considered. Fosdick and Hansen (5) have already shown that an enormous increase in lactic acid occurred when yeasts were added to cultures of lactobacilli. Furthermore, there are many variants among the oral lactobacilli (6), and our experience showed that the S forms produced acid more readily and in greater concentration than the R types. This correlation between fermentative ability and colony forms was recently established by Tracy (7) with another species of lactobacillus, *L. plantarum*. Hence, it would seem possible that symbiotic relationships and types of lactobacilli might influence the amount of acid produced by the organisms of the saliva. This possibility suggests the interesting speculation whether the amount of acid produced by the oral flora might not be a more accurate index of caries activity than numbers of lactobacilli as illustrated by the many exceptions to the production of significant amounts of acid in 48 hours or even longer periods by specimens of saliva with varying numbers of lactobacilli. This matter is of special interest since there is little information on the rate of cavity formation in terms of num-

bers or varieties of acidogenic bacteria in the mouth. An extensive program to test this point is being conducted and will be reported at a later time.

SUMMARY

Changes in color in selected time intervals of brom-cresol-green glucose or lactose infusion agar (pH 5.0) in "shake" tubes after inoculation of 0.1–0.2 c.c. of saliva were correlated with quantitatively estimated numbers of lactobacilli in the respective specimens. It was found possible to select by negative color change in 48 hours with 4 per cent error those specimens having less than 1000 lactobacilli per c.c. of saliva, but it became increasingly difficult to interpret color shifts directly in terms of lactobacilli in the saliva. The possibility of direct application in the study of dental caries is discussed.

REFERENCES TO LITERATURE

(1) Rodriguez, F. E., *J. A. D. A.*, 17: 1711, 1930.
(2) Hadley, F. P., *J. D. Res.*, 13: 415, 1933.
(3) Jay, P., *Bacteriology and Immunology of Dental Caries* in *Dental Science and Dental Art* edited by Gordon, S., (Philadelphia, Lea and Febiger, 1938).
(4) Boyd, J. D., Zentmire, Z. and Drain, C. L., *J. D. Res.*, 13: 433, 1933.
(5) Fosdick, L. S. and Hansen, H. D., *J. A. D. A.*, 23: 401, 1936.
(6) Hadley, F. P., Bunting, R. W. and Delves, E., *J. A. D. A.*, 17: 2041, 1930.
(7) Tracy, R. L., *J. Bact.*, 33: 467, 1939.

TRAUMATIC INJURIES IN THE JAWS OF INFANTS

JOSEF WEINMANN AND RUDOLF KRONFELD[1]

Foundation for Dental Research, Chicago College of Dental Surgery, Chicago, Ill.

It has recently become known that traumatic tissue injuries in human teeth are far more common than was previously believed. These injuries were found in erupted teeth and were attributed to excessive occlusal stress produced by various factors. In the systematic examination of sections through the jaws of many infants, ranging in age from the intrauterine period to several years after birth, numerous evidences of traumatic injuries were observed in bone sutures, in unerupted tooth germs, and in erupting teeth. For the sake of clarity these findings have been divided into preeruptive and posteruptive changes. The present paper deals with the *preeruptive* phase traumatic tissue changes in sutures and in unerupted tooth germs. The findings associated with erupted deciduous teeth will be reported in a later publication.

HISTOLOGIC FINDINGS

Sutures. *Fig. 1* shows a general view of a horizontal section through the upper jaw of an infant that died at the age of 18 days. This specimen was originally shown in a publication by Logan.[2] The germs of the deciduous incisors, canines, and molars are visible, as well as the germs of the permanent first incisors and of the permanent canines. Between the right and left first incisors the intermaxillary suture runs fairly straight in a sagittal direction; it is bordered by even bone surfaces in the anterior portion, whereas the palatinal portion shows a complicated serrated outline, caused by alternate deep indentations and sharp projections of the 2 premaxillary bones. In several places opposing spicules of bone come very close together. Such areas of near contact were found in the anterior portion, in the middle, and

[1] Deceased.

[2] *J. A. D. A.*, **22**: 20–21, 1935.

at the palatine end of the suture. A higher magnification from this specimen (*fig. 2*) shows, in the upper portion that the normal width of the suture is preserved. In the lower portion, 2 opposing spicules of bone project toward each other in such a way that the intervening connective tissue of the suture is compressed and hyalinized. Most of the nuclei have not stained in this area and the entire picture resembles closely the typical areas of early trauma found in the periodontal membrane of erupted teeth as a result of excessive occlusal

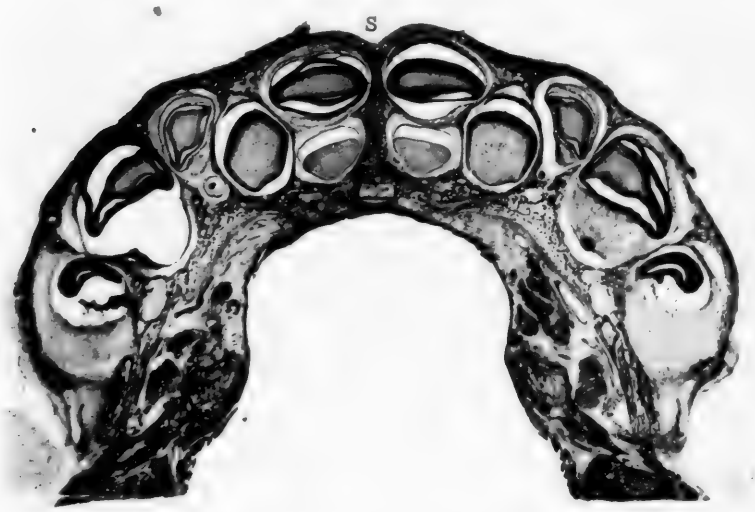


FIG. 1. Horizontal section through upper jaw of an infant that died at age of 18 days. S, median palatine suture. (Logan, *J. A. D. A.*, 1935.)

stress. A further similarity between this injury of the suture and traumatic injuries of the periodontal membrane is the beginning of undermining resorption, indicated by the presence of osteoclasts in the intact connective tissue adjacent to the injured area.

Similar areas of injury in the median suture were also observed in frontal sections through slightly older jaws. *Figs. 3* and *4* show the findings in a jaw of an infant of 9 months of age. Again there is a marked irregularity in the width of the suture; in several places the

opposing bone spicules are almost in contact, separated only by a thin layer of compressed connective tissue (*fig. 4*). In these areas there is rather extensive resorption of the bone surface in the vicinity of the injured connective tissue.

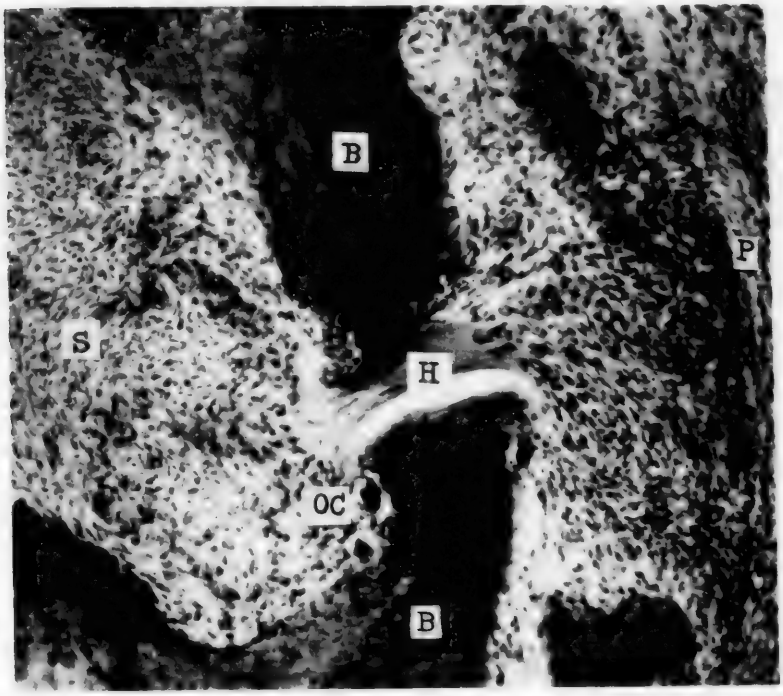


FIG. 2. Higher magnification of area marked by square in *fig. 1*. S, connective tissue of suture; B, B, bone spicules on opposite sides of suture; H, compressed, hyalinized connective tissue; OC, osteoclasts; P, periosteum.

The findings shown in *figs. 3* and *4*, though less pronounced than those in *figs. 1* and *2*, are important because they occurred in the jaw of an infant of 9 months, so that any possible birth damage to the bones of the skull and face may be safely excluded.

Tooth Germs.– *Fig. 5* shows a labiolingual section through the deciduous and permanent second incisor germs of an infant of 4 months. The 2 tooth germs lie in a common bony crypt, surrounded

by connective tissue of the tooth follicle and confined on the labial and lingual sides by thin plates of bone. A higher magnification (*fig. 6*) of

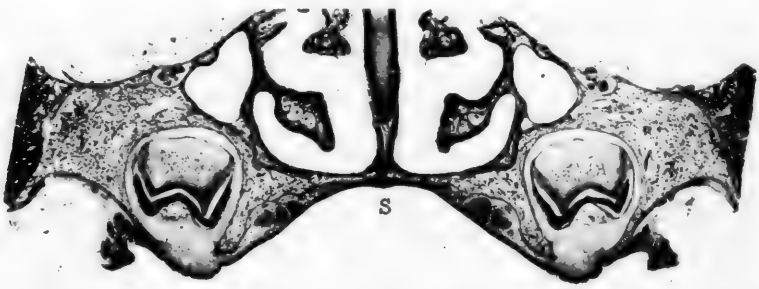


FIG. 3. Frontal section through upper jaw of infant of 9 months. Region of second deciduous molars. S, median palatine suture. (Logan and Kronfeld, *J. A. D. A.*, 1933.)

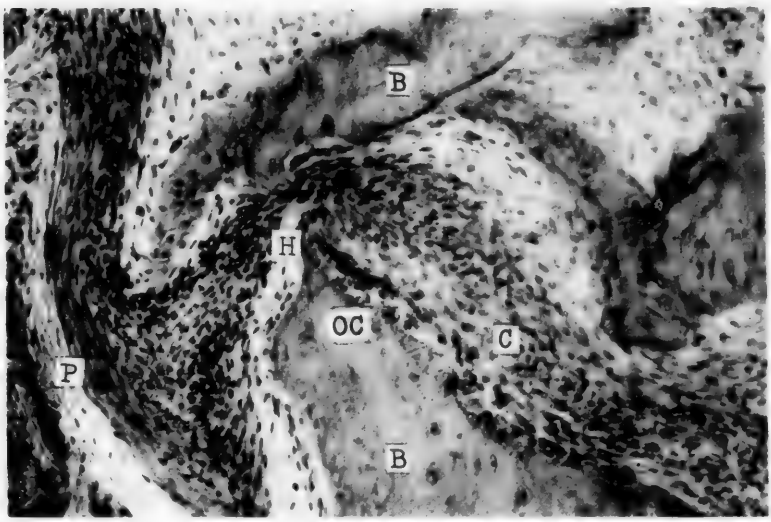


FIG. 4. Higher magnification of the median palatine suture in *fig. 3*. C, connective tissue of suture; B, B, bone spicules on opposite sides of suture; H, compressed, hyalinized connective tissue; OC, osteoclasts; P, periosteum.

the open root end of the deciduous incisor germ (area marked with a square in *fig. 5*) reveals that the tooth germ in this area is in very close contact with a prominent portion of the bone. The intervening

connective tissue is compressed and hyalinized. At the opposite side of the root end the distance between tooth and bone is not noticeably altered.

A similar condition was observed at the root end of an unerupted deciduous mandibular second incisor of an infant of 9 months of age. *Fig. 7* shows a general view of the erupting deciduous first and second incisors. The root of the latter has been forced against the bone of the tooth crypt on the mesial side causing compression of the connec-

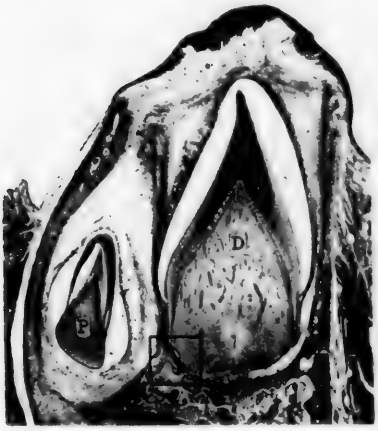


FIG. 5

FIG. 6

FIG. 5. Labiolingual section through the mandible and the deciduous and permanent second incisors of an infant of 4 months. D, deciduous tooth germ; P, permanent tooth germ.

FIG. 6. Higher magnification of area marked by a square in *fig. 5*. P, pulp; D, dentin; HS, Hertwig's sheath; B, bone; H, compressed, hyalinized connective tissue between root and bone.

tive tissue of the tooth follicle. The uncalcified extremity of the root is slightly deformed, and Hertwig's sheath somewhat displaced upward and into the pulp tissue (*fig. 8*). Above the area of contact, the bone surface is covered with osteoclasts.

A type of growth injury of a deciduous tooth in which possible occlusal stress and growth of the permanent tooth acted jointly as causative factors is illustrated in *figs. 9* and *10*. The specimen shows a mesiodistal section through the mandibular deciduous canine, first

deciduous molar, and permanent canine germ of a child of $1\frac{1}{2}$ years of age. The first premolar germ is not yet calcified; it lies between the roots of the deciduous molar. The distal root of the latter is straight and normal. The apical end of the mesial root, however, is sharply curved toward the distal side. A higher magnification of this root end (*fig. 10*) reveals a narrow, compressed periodontal membrane on

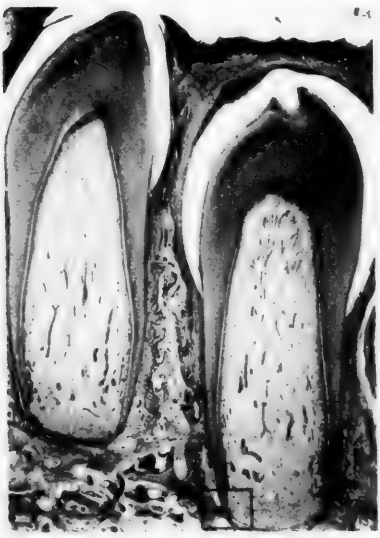


FIG. 7

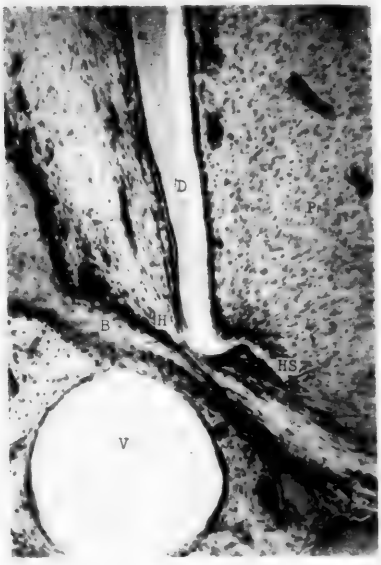


FIG. 8

FIG. 7. Mesiodistal section through a lower deciduous first incisor (erupting) and second incisor (unerupted). Age, 9 months.

FIG. 8. Higher magnification of area marked with a square in *fig. 7* (root end of second incisor). P, pulp; D, dentin of root end; HS, Hertwig's sheath; B, bone; H, compressed, hyalinized connective tissue between root and bone; V, vein.

the convex side of the curved root and a widened periodontal space on the upper, concave side. Hertwig's sheath is deformed similarly to the condition already shown in *fig. 8*. This deformity of the root end is probably the combined result of occlusal pressure, which tends to force the tooth into its alveolus, and of the encroachment of the growing permanent tooth germ.

COMMENTS

The growth injuries described in this report can be classified into 3 groups: Injuries of the intermaxillary suture; injuries to the connective tissue and bone surrounding the tooth germ; and injuries to the tooth germ proper. The first type, injuries to the suture between the maxillae, can be explained in 2 ways: either the bones forming the suture were subjected to some kind of shift or relative displacement,

FIG. 9. Mesiodistal section through mandible deciduous (III) and permanent (3) canine and first deciduous molar (IV). Age, 1½ years.

or spicules from the bones grew toward each other at the expense of the intervening connective tissue.

The first of these possibilities, namely, a shifting of the 2 maxillary or premaxillary bones toward each other or parallel to each other, can be assumed as a result of external traumatic influence or of uneven growth of the halves of the face or jaw. The second possibility is that on diametrically opposite sides of a suture, bone grows until the space between the two is greatly reduced. Normally, apposition of bone along the suture line is accompanied by resorption or by moving

apart of the bordering bones, so that the normal suture relations are maintained (1). If either the bones fail to move apart, or if bone grows more extensively in one area than in another, the opposing bone spicules approach each other. If now minute movements of the bones occur, the result is the same as when root surface and bone come in contact, compression and hyalinization of the intervening connective tissue (*figs. 2* and *4*). This compression is later followed by

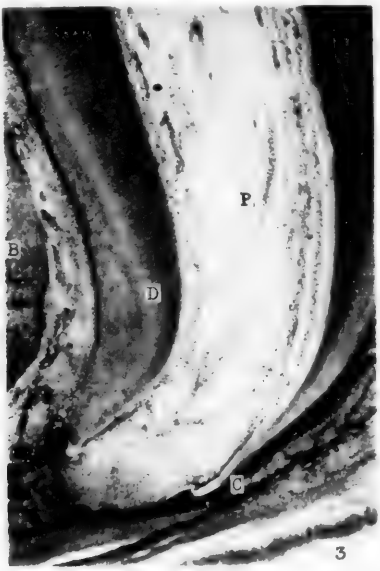


FIG. 10. Higher magnification of the mesial root of the first deciduous molar shown in *fig. 9*. P, pulp; D, dentin curved at the root end; B, bone; C, compressed periodontal connective tissue; 3, germ of permanent cuspid.

repair, which is initiated by undermining resorption of the bone in the area of connective tissue damage. A similar process was recently demonstrated by Brodie (2) in his study of the growth of the skull. Sometimes the growing bones fail to move apart at the same time. The reason for this is unknown. Subsequent examination of such individuals shows complete recovery and readjustment.

The second type of injury is compression of the connective tissue between the unerupted tooth germ and the wall of the surrounding

bony crypt. Normally the bone of the crypt responds to an increase in the size of the tooth germ by resorption. In this way, the distance between the surface of the enamel organ, or later of the tooth germ, and the bone remains fairly wide and uniform. If for some reason, while the tooth germ continues to grow, any area of the crypt fails to respond to the resorption, then the space between bone and tooth germ is reduced, and finally the intervening connective tissue in a circumscribed area is compressed (*figs. 6* and *8*). Ultimately the outcome is probably the same as in the suture injury, namely, undermining bone resorption and restitution of the normal thickness of the periodontal space.

The third possibility is injury to the tooth germ proper. A traumatic deformation of Hertwig's sheath, as shown in *fig. 8*, may result in a minor deformity of the root, and possibly also of the crown, if it occurs earlier in the development of the tooth. A similar deformity of a deciduous tooth germ can be caused by the pressure of the growing and moving germ of a permanent tooth, especially if occlusal pressure forces the deciduous tooth against the permanent tooth germ, as shown in *figs. 9* and *10*. Under such circumstances traumatic injuries may also be found in permanent tooth germs as recently shown by Grimmer (3).

The preeruptive changes described in this report are rather uncommon, having been found only in a few of many jaws examined. Moreover, it can be assumed that these tissue damages are usually repaired and the normal relations restored, as evidenced by the observation of undermining resorption in almost every instance of injury. The only possible persisting damage may be abnormalities in the configuration of the root, such as indentations in the root surface or deflections of the dentino-cemental junction. Diamond (4) has suggested this in his discussion of the factors which may be responsible for acquired variations in the shape and size of teeth. He mentions intraosseous pressure as a possible mechanical interference during the developmental period.

SUMMARY

Histologic examination of jaws from intrauterine life to several years of age revealed in several instances traumatic changes between

adjoining bones of sutures, and between the tooth germs and their bony crypts. As explanation of such pathologic occurrences, uneven growth of opposing hard tissues and minor shifts of the growing parts was assumed. There are indications that these traumatic conditions usually are repaired, with the possible exception of changes affecting the normal contour of the growing root.

REFERENCES TO LITERATURE

(1) Noyes, F. B., *Angle Orthodont.*, 4: 123, 1934.
(2) Personal Communication.
(3) Grimmer, E. A., *J. D. Res.*, 18: 267, 1939.
(4) Diamond, M., *Dental Anatomy*, 2nd Ed. (New York, Macmillan Co.: 1935) p. 226.

AN EVALUATION OF ROOT RESORPTION OCCURRING DURING ORTHODONTIC TREATMENT[1]

CHARLES E. RUDOLPH, D.D.S.

Division of Dental Research and Graduate Education, University of Minnesota, Minneapolis, Minn.

Since we believe that, other things being equal, root resorption will occur more frequently in patients treated orthodontically than in individuals who have not received orthodontic treatment, it has been our aim to discover if this belief is based upon fact and, if so, to what extent this premise can be applied for the purpose of prognosticating root resorption.

Stimulus to this investigation was given by Ketcham who urged "all orthodontists to secure accurate radiographic surveys of all cases before treatment, and to recheck them often, compiling results so that many accurate data may be secured" (1).

A preliminary report was made in 1936 of the incidence of root resorption developing in patients receiving orthodontic treatment in the undergraduate clinic in the School of Dentistry of the University of Minnesota (2). Serial roentgenograms of 439 orthodontic patients, whose ages ranged from 7 to 21 years, were studied. None of these patients showed any evidence of root resorption before orthodontic treatment was instituted. At the end of the first year of treatment, 49 per cent of the 439 patients showed root resorption; and at the end of two years of treatment, 75 per cent of the remaining 277 patients demonstrated root resorption.

For purposes of comparison the roentgenograms of 4,560 patients with no history of orthodontic treatment were studied. The individuals in this group ranged in age from 7 to 79 years, and the incidence of root resorption in this group was 13 per cent. However, when the 739 patients ranging from 7 to 21 years of age, the age group compa-

[1] Read at the 18th General Meeting of the International Association for Dental Research, Philadelphia, Pa., March 13–14, 1940 (*J. D. Res.* 19: 295, 1940).

rable to that of the orthodontic patients, were segregated 38, or 5 per cent, demonstrated root resorption. All of these 38 patients were over 13 years of age. In other words, of the 739 cases with no history of orthodontic treatment, no patient 13 years of age, or under, showed root resorption.

In the study of the relationship between orthodontic treatment and root resorption a number of related factors must be considered. The most important of these, in our opinion, are age at onset of treatment, duration of treatment, and, possibly, sex. It has been possible to ascertain these factors, together with the radiographic examinations, from the records of 513 orthodontic patients treated in the under-

TABLE I

Percent incidence of root resorption with duration of treatment

AGE AT ADMISSION	NUMBER TREATED	YEARS FOLLOWED						
		1	2	3	4	5	6	7
Up to 8	46	4	16	39	57	94	100	100
9 Years	47	6	30	66	86	94	97	100
10 "	71	25	57	79	98	100	100	100
11 "	90	39	75	93	100	100	100	100
12 "	110	49	81	97	99	100	100	100
13 "	76	61	93	97	99	100	100	100
14 "	38	63	95	100	100	100	100	100
15 "	21	67	89	100	100	100	100	100
16 and over	14	57	100	100	100	100	100	100

Data from Undergraduate Orthodontic Clinic, School of Dentistry, University of Minnesota.

graduate clinic of the School of Dentistry of the University of Minnesota from January 1928 to January 1940. Of the 513 patients, 219 were male and 294 were female. Dental roentgenograms were obtained before orthodontic treatment was started and at approximately one year intervals during the course of treatment. These films were analyzed for the occurrence of root resorption by members of the orthodontics staff. No orthodontic patient has been excluded from the study for any reason other than incomplete roentgenological data. The type of orthodontic treatment as carried out by the undergraduate student in this clinic consists of a labial arch appliance with fixed abutment attachments and bands with ligatures on other teeth

needing correction. Adjustments to this appliance were made at weekly intervals.

The data thus collected was divided according to sex and age at onset of treatment and the incidence of root resorption relative to the duration of treatment was tabulated. The accumulative data was arranged in tables and the per cent incidence of root resorption was computed. In the use of accumulative data it is rightly assumed that once root resorption has occurred, loss of tissue is permanent.

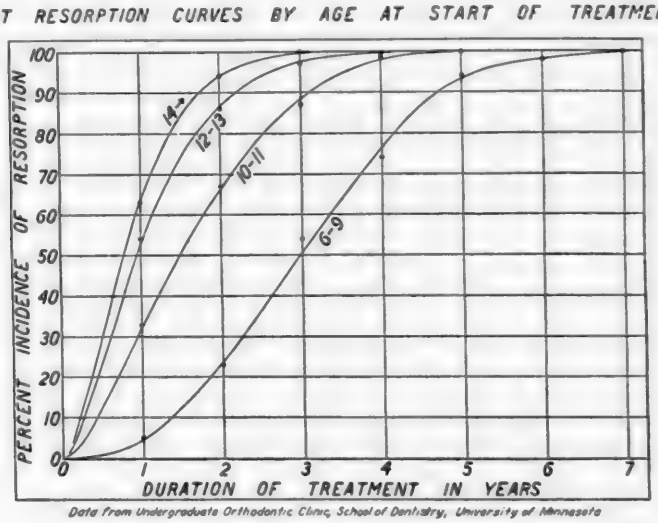


FIG. 1

In Table I it is clearly demonstrated that per cent incidence of root resorption progresses with each year of treatment and with increased age at onset of treatment. For example, in the group of 47 patients who were 9 years of age at the onset of treatment, the per cent incidence of root resorption for each successive year of treatment is 6, 30, 66, 86, 94, 97, and 100 respectively. In contrast to this 9 year old group, the per cent incidence of root resorption for each successive year of treatment in the 110 patients in the 12 year old group is 49, 81, 97, 99, and 100. Thus, in the 12 year old group it would be expected that evidence of root resorption would appear at a

point which is considerably earlier in the treatment than in the case of the 9 year old group. Still earlier evidence of root resorption is found in the group of 21 patients who were 15 years of age at the onset of treatment. This is shown in Table I as 67, 89, and 100 per cent respectively for each succeeding year of treatment.

TABLE II

Percent incidence of root resorption with duration of treatment

AGE AT ADMISSION	NUMBER TREATED	YEARS FOLLOWED						
		1	2	3	4	5	6	7
Male								
Up to 8	23	4	11	27	45	86	100	100
9 Years	23	0	15	47	79	86	92	100
10 "	27	19	43	63	94	100	100	100
11 "	35	29	71	96	100	100	100	100
12 "	48	38	75	95	97	100	100	100
13 "	31	68	90	96	96	100	100	100
14 "	19	63	94	100	100	100	100	100
15 "	7	71	100	100	100	100	100	100
16 and over	6	33	100	100	100	100	100	100
Female								
Up to 8	23	4	20	50	67	100	100	100
9 Years	24	12	43	81	90	100	100	100
10 "	44	30	65	88	100	100	100	100
11 "	55	45	77	91	100	100	100	100
12 "	62	58	86	98	100	100	100	100
13 "	45	56	95	98	100	100	100	100
14 "	19	63	95	100	100	100	100	100
15 "	14	64	85	100	100	100	100	100
16 and over	8	75	100	100	100	100	100	100

Data from Undergraduate Orthodontic Clinic, School of Dentistry, University of Minnesota.

Fig. 1 shows the per cent incidence of root resorption in the 513 orthodontic patients grouped according to age as follows: 6 to 9, 10 and 11, 12 and 13, and 14 and over. We observe that 50 per cent of the patients in each group may be expected to demonstrate the phenomena after 3 years of treatment, 1½ years of treatment, 1 year of treatment, and before the end of the first year of treatment respectively.

Due to the small number of cases in each age group, statistical methods for determining sex differences were not employed. Table II shows per cent incidence of root resorption according to sex, age at onset of treatment, and duration of treatment.

SUMMARY

From the data which has been obtained from the study of 513 patients receiving orthodontic treatment in the undergraduate clinic it is apparent that such treatment is less hazardous to the root structures when it is initiated at an early age. Moreover, the younger the patient at the onset of treatment, the more prolonged can the treatment be without permanent loss of root tip structure.

Grateful acknowledgement is given to Dr. Alan E. Treloar, Department of Preventive Medicine and Public Health, University of Minnesota for his valued suggestions and assistance in the treatment of the data and to Dr. Charlotte Fisk, Division of Dental Research and Graduate Education, University of Minnesota for her cooperation.

REFERENCES TO LITERATURE

(1) KETCHAM, ALBERT H., *J. A. D. A.*, 14: 1577, 1927.
(2) RUDOLPH, C. E., *J. A. D. A.*, 23: 822, 1936.

THE EFFECTS OF APPLICATION OF VITAMIN D TO DENTIN IN DOGS[1]

P. S. FAILLO, D.D.S., AND C. I. REED, PH.D.

WITH THE TECHNICAL ASSISTANCE OF J. FELDMAN AND T. R. SHERROD

Department of Physiology, University of Illinois, Chicago, Ill.

The investigations upon which this paper is based were prompted by a report by Weinmann (1) that the application of vitamin D to cavities prepared in the teeth of dogs produced secondary dentin.

The relation of vitamin D to dental health has been the subject of lively controversy. The literature was recently reviewed by Mellanby and King (2), who believe that the problem of dental caries and peridental disease is fundamentally a problem of nutrition and that the tooth structure, as conditioned by nutrition, is of primary importance in immunity or susceptibility to caries. Fish (3), on the other hand, reported that high supplements of vitamin D and calcium carbonate fed to dogs did not increase the calcium content of the dentin, although there was increased opacity of bones to x-rays, indicating that the treatment did increase the calcium content of those tissues able to receive it. Dentin appears, then, to be devoid of a mechanism by which calcium may be deposited or withdrawn.

Fish further called attention to the fact that:

"when calcium salts are deposited in the dentin in the form of translucent zones in response to local injury, they occupy the lumina of the tubules and block them off; they are not incorporated in the matrix. Similarly the slight increase in calcium content observed in human teeth in the first few years after eruption is associated with an anatomical reduction in the diameter of the tubule.

"The suggestion that the exhibition of vitamin D affects the incidence and progress of caries, appears to rest on two assumptions: 1. That the exhibition of the vitamin will increase the calcium content of the dentin. 2. That an increased calcium content of the dentin will prevent caries."

---

[1] This investigation was supported in part by a grant from the Nutrition Research Laboratories. Part of the material used was supplied by this firm and part by Mead Johnson Company.

Mellanby selected the dog as an experimental animal, largely because it could maintain good health under laboratory conditions and because the omnivorous diet closely resembles that of man. The objections to this animal as a subject for this type of experiment are that its denture is unlike that of man and caries is not commonly found. While the monkey would have been more suitable for overcoming these objections, its diet is vegetarian. Thus the dog, adopting human diet, is relatively immune to caries while in the native state.

The conflicting evidence serves to emphasize the complexity of the problem so that it is not germain to the present discussion to review further the literature except to call attention to the report by Van Huysen and Gurley (4) in which these authors contended that the process of cutting a cavity, the depth thereof, and the length of time it had existed affected the histological appearance of the pulp tissues, the predentin layer, the odontoblastic layer and the deposits of secondary dentin. These observations serve to emphasize the possibility that Weinmann may have misinterpreted the conditions actually observed.

EXPERIMENTAL PROCEDURE

Particular care was exercised in selecting healthy, young adult dogs with well-formed, fully developed teeth. After anesthetization with nembutal, cavities were prepared with a dental drill in the gingival third of the labial surfaces of both maxillary and mandibular incisors. The cavities were extended well down into the dentin.

Half of the cavities were treated, the other half serving as controls. Over 100 cavities were prepared in 15 dogs. Part of the control cavities were left open, others were packed with inactive ergosterol and sealed with dental cement (zinc phosphate), and others were sealed with cement without any treatment.

The experimental cavities were sealed with cement after packing with activated ergosterol in varying dosages. Dry crystalline material was used in some, in others a concentrated ether solution of calciferol was placed in the cavity and the ether allowed to evaporate. In units of vitamin D the dosages per dog ranged from 10,000 units to 500,000 units. The after period varied from 4 to 6 months, at the expiration of which the animals were killed by intracardiac injection of ether. The jaws were preserved in formalin for histological examination.

Samples of 12 organs were taken from most of the animals for determination of the calcium and phosphorus content. The blood calcium was followed in a number of animals.

In the earlier experiments the conception of the problem was so conditioned by Weinmann's findings that the possibility of anything other than a local action of the vitamin was not taken into account. It was only after a chance observation on a blood sample revealed

hypercalcemia of 15.00 mg. per 100 cc. that it was realized that the vitamin was being absorbed and exerting a systemic effect just as when fed or injected.

The physiological and pathological actions of vitamin D have been discussed elsewhere (5) in much greater detail than is possible in this paper. It is only necessary here to call attention to the fact, now well established, that this vitamin in any form can be readily absorbed from any site.

The variable behavior of the calcemia in the two dogs is in line with that observed in all others hence only two protocols are included in Table I. The picture is consistent with that observed in any animal heavily D-vitaminized. The calcemic level will fluctuate unaccountably over a long period. The low initial calcemia is not infrequently observed in dogs received from the pound and is a condition which is only slowly corrected in the institutional kennels unless special treatment is instituted. Dogs that have been D-vitaminized recover normal calcemia very slowly. The slow development of hypercalcemia is comprehensible when one realizes that, regardless of the state in which the vitamin was put into the cavity, it was in solid form when finally sealed in. Therefore, to become effective systemically it must be dissolved by some agency and carried through the tubules to the pulp cavity. This was a rather surprising result.

The calcemic changes would not be so striking, taken alone. But with the analyses of tissues for content of calcium, when compared to values for normal dogs previously obtained in this laboratory (5) show that there was a general tendency to an increase in calcium. This was not consistently observed in every tissue but the values tended be higher. The results for the two dogs in Table I are shown in Table II. The others are omitted to conserve space. For comparison mean normal values are included.

Bearing in mind the possible variability of the absorption of the vitamin from dental cavities, too much stress should not be laid on quantitative differences among the animals. In general, they all behaved systemically like animals receiving a mild overdose of vitamin D.

There was commonly degeneration of the pulp in all treated teeth, but this could not be correlated quantitatively with the dose of vita-

min D, so its significance is open to question. The most important observation, however, was that in all preparations, whether control or

TABLE I

Blood calcium and inorganic phosphorus

	Ca	INORG. P	
	(mgm. per 100 cc.)	*(mgm. per 100 cc.)*	
DOG F 1			
Initial	8.6	4.9	Treated
37th day	9.4	4.3	
51st "	9.0		
72nd "	22.4	3.8	
101st "	11.9	3.3	
115th "	11.2	3.7	
122nd "	10.7	4.2	
129th "	11.0	4.0	
136th "	9.6	4.1	
143rd "	11.1	3.8	
150th "	12.6	3.4	
157th "	11.8		
164th "	11.0	3.8	
185th "	10.9	3.7	
191st "			Killed
DOG D 2			
Initial	6.3	3.8	Treated
28th day	7.2	3.8	
42nd "	7.6	3.7	
63rd "	8.8	3.6	
92nd "	10.8	4.4	
106th "	9.4	3.8	
113th "	9.4	4.2	
120th "	10.0	4.1	
127th "	9.5	4.1	
134th "	10.8	4.3	
141st "	11.0	5.0	
148th "	10.9	4.5	
156th "	11.3	4.3	
177th "	9.7	4.2	
189th "			Killed

treated, there were layers of secondary dentin deposited on the wall of the pulp cavity opposite the prepared cavity (*figs. 1–4*). In many cases this was thicker and longer in the control teeth than in those

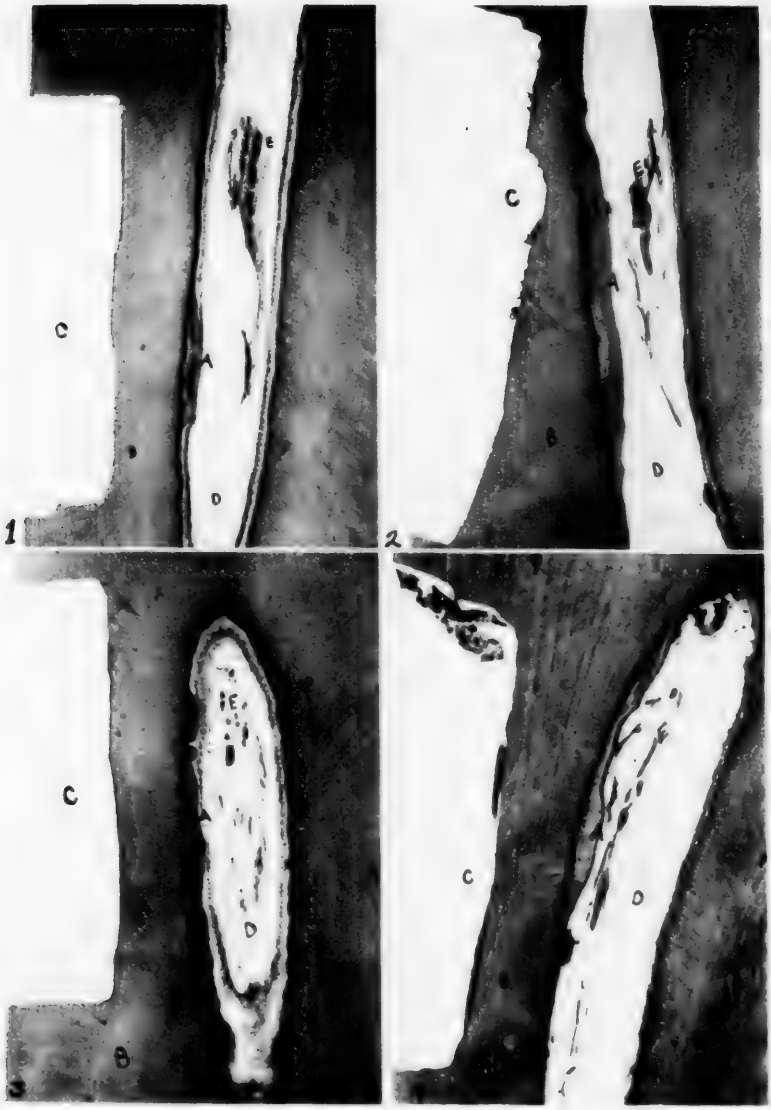


FIG. 1. Cement Control, No. 3402. Dog tooth (×15), 191 days. A, secondary dentin layer; B, dentin; C, cavity; D, pulp chamber; E, remnants of pulp.

FIG. 2. Cement control, No. 3407. Dog tooth (×15), 191 days. A, secondary dentin layer; B, dentin; C, cavity; D, pulp chamber; E, remnants of pulp.

FIG. 3. Irradiated Ergosterol, No. 3406. Dog tooth (×15), 191 days. A, secondary dentin layer; B, dentin; C, cavity; D, pulp chamber; E, remnants of pulp.

FIG. 4. Irradiated Ergosterol, No. 3406. Dog tooth (×22.5), 191 days. A, secondary dentin layer; B, dentin; C, cavity; D, pulp chamber; E, remnants of pulp.

treated. Therefore, the deposits of secondary dentin observed in all preparations were formed as a result either of the stimulus of the cavity preparations or of the cementing material and not by the local calcifying action of the vitamin D. There is much evidence in the literature that either zinc phosphate cement alone, cavity preparation alone or a combination of these may stimulate the formation of

TABLE II

Calcium content of tissues

	F 1	D 2	MEAN NORMAL
Adrenal	149*		41
Aorta	149	108	67
Brain	95		63
R. Vent.	32	44	29
L. Vent.	49	37	19
Kidney	112	92	64
Liver	75	59	19
Lung	133	103	87
Muscle	26		21
Skin	55	53	25
Spleen	75	60	15
Thyroid	270	190	109

* All values in mgm. per 100 gms. of dry tissue.

secondary dentin. These findings support the views of Van Huysen and Gurley, but do not confirm those of Weinmann.

SUMMARY

The topical application of vitamin D in prepared cavities in the teeth of healthy dogs results in systemic effects manifested by hypercalcemia and hypercalcia in soft tissues.

Deposits of secondary dentin are as readily formed on the wall of the pulp cavity in control teeth as in those treated with vitamin D. Therefore, these deposits are not due, as Weinmann thought, to a local calcifying effect of the vitamin.

The histological preparations were made by the late Dr. R. Kronfeld of The Foundation for Dental Research of the Chicago College of Dental Surgery, and we wish to acknowledge our deep appreciation of his cooperation.

REFERENCES TO LITERATURE

(1) Weinmann, J., Action of vitamin D by local application in dental cavities. *J. D. Res.* **13:** 220, 1933.

(2) Mellanby, M. and King, J. D., Vitamins and dental caries. *Ergebn. d. Vitamin- und Hormonforch.* **2:** 1, 1939.

(3) Fish, E. W., The effect of vitamin D on the calcium content of the dentine. *J Physiol.* **84:** 272, 1935.

(4) Van Huysen, G. and Gurley, W. B., Histologic changes in the teeth of dogs following preparations of cavities of various depths and their exposure to oral fluids. *J. A. D. A.* **26:** 87, 1939.

(5) Reed, C. I., Struck, H. C. and Steck, I. E., *Vitamin D. Chemistry, physiology, pharmacology, pathology, experimental and clinical investigations.* (Chicago, University of Chicago Press: 1939.)

THE EFFECT OF CASTRATION AND HYPOPHYSECTOMY ON THE GINGIVAE AND ORAL MUCOUS MEMBRANES OF RHESUS MONKEYS[1]

DANIEL E. ZISKIN, D.D.S., AND S. N. BLACKBERG, PH.D., M.D.[2]

School of Dental and Oral Surgery and Department of Pharmacology, Columbia University, New York, N. Y.

The material for this report is taken from studies on 3 groups of rhesus monkeys. Group I consists of 10 normal female monkeys of different ages and weights from which sections of gingival tissue were taken previous to any experiments. This represents the normal control series. Group II consists of 7 monkeys—4 females and 3 males. These animals were castrated and subsequently observed over varying periods of time before autopsy. No treatment other than castration was given. Group III consists of 5 hypophysectomized female monkeys. No treatment other than the hypophysectomy was given.

METHOD

In the control group (I), the monkeys were anesthetized with ether and an interdental papilla was excised from each. Both alveolar and areolar gingivae were included in the biopsy (Table I). In the castrate group (II), large sections of alveolar and areolar gingivae, as well as sections from the mucous membrane of the cheek, were taken at autopsy (Table II, A and B). In the hypophysectomized group (III), the heads were preserved in formalin for several weeks before removal of the tissues (Table II, C). The tissues were prepared for study by fixation in Bouin's solution, with clearing in the usual way. They were then embedded in paraffin and sectioned. H&E and Masson's trichrome stains were used in all cases. The sections were cut longitudinally from labial to lingual aspect of each interdental papilla.

DESCRIPTION OF MICROSCOPIC APPEARANCE OF TISSUE IN NORMAL CONTROL GROUP (10 ANIMALS)

These monkeys were untreated prior to the taking of gingival sections. The tissue as a whole stained deeply and, for the most part, uniformly.

[1] Read at The 18th General Meeting of the International Association for Dental Research, Philadelphia, Pa., March 13-14, 1940 (*J. D. Res.* **19:** 326, 1940).

[2] Now at University of Arkansas, School of Medicine, Little Rock, Ark.

The Alveolar Gingivae: The keratin layer, although thin in some animals and thick in others, was definite. Granulosa cells seen in human gingivae were absent. Some elongated, horizontally arranged cells were seen near the surface, but these did not resemble granulosa cells. The prickle cell layer was generally compact and well formed with uniform vesicular nuclei. Intercellular bridges were numerous and prominent. The basal cell layer appeared well formed and even, with 1 to 4 mitotic figures (sometimes more) in a section. Most of the epithelial pegs were slender and rounded; occasionally they were

TABLE I

Normal group

MONKEY NO.	SEX	WEIGHT	SITE OF BIOPSY	KERATIN	
				Alveolar	Areolar
		gm.			
205	♀	5,300	U. L. #1	++	0
208	♀	7,350	U. L. #1	+++	0 to ±
209	♀	5,450	U. L. #1	±	0
210	♀	5,500	U. L. #4	±	0
269	♂	5,200	U. L. #1	++	0
271	♂	5,100	U. R. #1	++	0
272	♂	3,850	U. L. #1	+++	0
278	♂	1,100	U. L. #1	++	0
285	♂	8,900	U. L. #1	+	0 to ±
286	♂	4,050	U. R. #1	+	0

Degree of thickness of keratin layer from: 0 = Absent to +++ = heavy keratin layer. Method of identifying papilla: between centrals = central papilla; between central and lateral = #1; between lateral and cuspid = #2; etc.

pointed. In the stroma the blood vessels were normal with the exception of occasional fields of congestion. Slight areas of inflammatory exudate were seen near the tip in most cases.

The Areolar Gingivae: The keratin layer was absent. In 2 of the 10 animals small areas of keratin were seen but these did not constitute a definite layer. Granulosa cells were not seen. The prickle cell layer was compact in the region nearest the alveolar gingivae, but at higher levels, approaching the muco-buccal fold, it became loose, with large vari-sized vesicular nuclei. The intercellular bridges were not well defined. The basal layer was usually well formed and even, showing fewer mitotic figures than the alveolar gingivae. The epithel-

ial peg pattern also differed from that of the alveolar gingivae in that the pegs were broader, shorter, and rounded in such a way as to give the whole the appearance of a scalloped border. Some areas appeared flat and showed no pegs at all. In the stroma, the blood vessels were

TABLE II

MONKEY NO.	SEX	WEIGHT	SITE OF BIOPSY	KERATIN		DURATION
				Alveolar	Areolar	
			A. Female castrates			
		grams				*days*
213	♀	About 3,850	U. L. ✱1	+	0	73
214	♀	About 3,850	U. L. ✱1	+	0	73
253	♀	3,900	U. L. ✱1	++	0	240
321	♀	3,750	U. L. ✱2	±	0	120
			B. Male castrates			
280	♂	1,750	U. L. ✱2	+	+	155
297	♂	3,600	Upper Central	++	++	150
303	♂	3,800	Upper Central	+	+	150
			C. Hypophysectomized (Complete)			
238	♀	3,850	U. R. ✱1	0	0	47
241	♀	3,300	U. L. ✱1	±	0	10
243	♀	3,900	U. L. ✱1	0	0	38
244	♀	3,900	U. L. ✱1	0	0	60
246	♀	3,900	U. L. ✱1	±	0	53

Degree of thickness of keratin layer from: 0 = Absent to +++ = heavy keratin layer. Method of identifying papilla: between centrals = central papilla; between central and lateral = ✱1; between lateral and cuspid = ✱2; etc.

normal, with the exception of occasional fields of congestion. Inflammatory exudate was rarely seen.

DESCRIPTION OF MICROSCOPIC APPEARANCE OF TISSUE IN CASTRATE GROUP (7 ANIMALS)

Ute 1 Females:

The tissue as a whole did not stain as deeply nor as evenly as the normal tissue, and assumed a greyish lavender color. Noteworthy was the tendency of the epithelial tissue to fragment and strip off

in sectioning. The stripping off occurred often as a separation of the upper layers of the epithelium from the basal layer or across the center of the tissue. Most of the stripping took place in the areolar gingivae and the upper layer was frequently lost in sectioning. This degeneration seemed to be more acute in the long-time castrates (*fig. 1*).

The Alveolar Gingivae: In some cases the keratin layer was definite, well formed, but thin; in others, keratin was seen only in spots with no definite layer formation. Granulosa cells were absent here as in

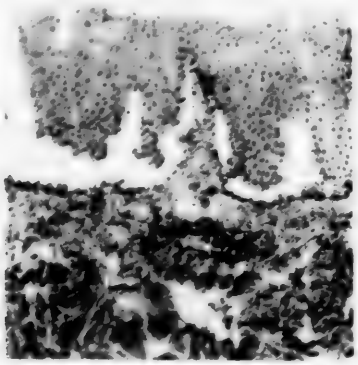


FIG. 1. MY #258 ♀. Hypophysectomized (complete removal) 47 days. No other treatment. Shows chiefly stripping off of surface epithelium. This tendency, while classed as artefact, appeared only in female castrates and hypophysectomized animals. (Piece of cheek. × 75.)

the control group. The prickle cell layer showed marked alteration (*fig. 2*). Instead of the typical horizontal arrangement, the cells were columnar-like in shape and disposed in vertical rows. The disappearance of nuclei was noted frequently and in some instances the tissue presented a moth-eaten aspect (probably lysis of the cells (*fig. 3*). Intercellular bridges were prominent. The basal cell layer was not as sharply demarcated as in the control group. The nuclei were smaller than normal and hyperchromatic. Mitosis was not markedly altered. The epithelial pegs were irregular both as to size and shape, some being pointed and some rounded, while elonga-

The Areolar Gingivae: Keratin and granulosa cells were absent. Near the surface the prickle cells appeared larger and flatter than in the deeper portion of tissue. The prominent intercellular bridges and arrangement of cells in vertical rows in the deeper layers resembled closely those seen in the alveolar gingivae. The cytoplasm appeared

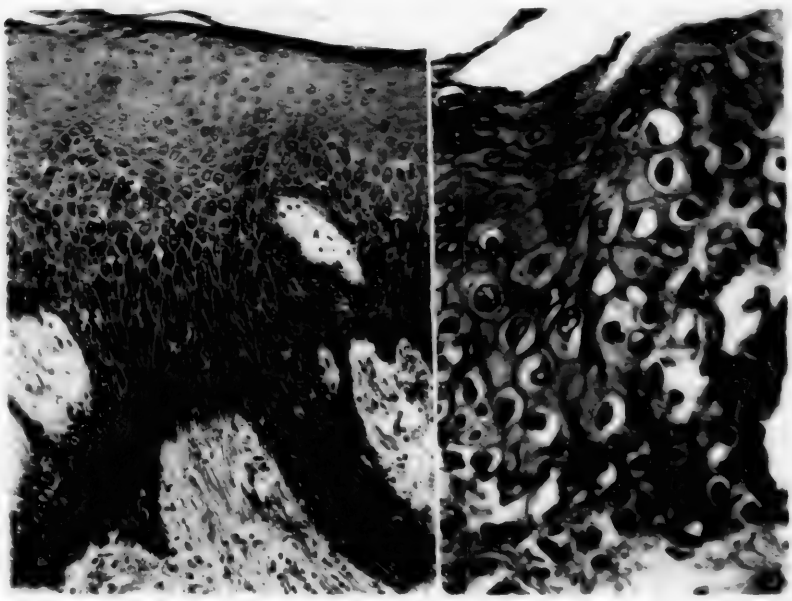


Fig. 2 Fig. 3

Fig. 2 MV ♦214 ♀ Castrated No other treatment 75 days Shows [illegible] altered arrangement of prickle cells, columnar in shape and disposed in vertical rows (Lower right ♦2 papilla low power alveolar gingiva

Fig. 3 MV ♦233 ♀ Castrated 240 Days No other treatment Shows [illegible] moth-eaten appearance of tissue, evidence of tissue degeneration. (Upper right ♦2 papilla areolar gingiva ×540)

granular. The basal cell layer was not as sharply demarcated as in the control group. The epithelial pegs were shorter than in the alveolar gingivae, and more flattened and rounded. The whole epithelial layer was thinner. In one section, cyst formations were seen in the prickle cell layer

In the stroma of both alveolar and areolar gingivae, the blood vessels were not much changed; more inflammatory exudate was seen near the crest of the interdental papilla than elsewhere. The collagen appeared stringy and loose; it stained more faintly than normal but the alteration was not impressive.

The 3 Males:

The tissue as a whole took a bright eosin stain. The keratin layer was definite and well formed, extending as a definite layer on the areolar gingivae as well as on the alveolar gingivae. Underneath the keratin in both gingivae, there was a zone of parakeratosis. The prickle cell layer was fairly normal in appearance, although in some areas a vertical arrangement of the cells, characteristic of the female castrates, was seen (*fig. 4*). The basal cell layer appeared normal. The epithelial pegs were somewhat pointed but not markedly so. Epithelial pearls and curls were seen.

There were no outstanding changes in the stroma, the blood vessels appearing normal, and inflammatory reactions not as marked as in the female castrate series. The most noteworthy change was the tendency toward hyperkeratinization (*figs. 5* and *6*).

DESCRIPTION OF MICROSCOPIC APPEARANCE OF TISSUE IN HYPOPHYSECTOMIZED GROUP (5 ANIMALS)

The tissue as a whole took a faint greyish lavender stain. Keratin was present in spots on the alveolar gingivae but was for the most part absent. There was none on the areolar gingivae. Granulosa cells were absent. The prickle cell layer showed marked evidence of degeneration. The cells appeared irregular in size and shape; vacuolization was seen in both the cytoplasm and nuclei. The intercellular bridges were poorly preserved. The tissue stripped off easily and much of it was lost in sectioning.

In 4 of the 5 monkeys studied, most of the superficial epidermal layers were missing and only the basal cell layer and a few layers of prickle cells remained. In some of the better preserved sections, the vertical arrangement of the prickle cells, characteristic of the female castrate group, could be seen (*fig. 7*). The peg pattern was not as pointed as in the female castrate group, but rounded or scalloped. The collagen took a fainter stain than the normal group. There were

no significant differences in the blood vessels, inflammatory reactions or mitosis to account for the extreme changes noted. The evidences of tissue degeneration (*fig. 8*) were more marked in the hypophy-

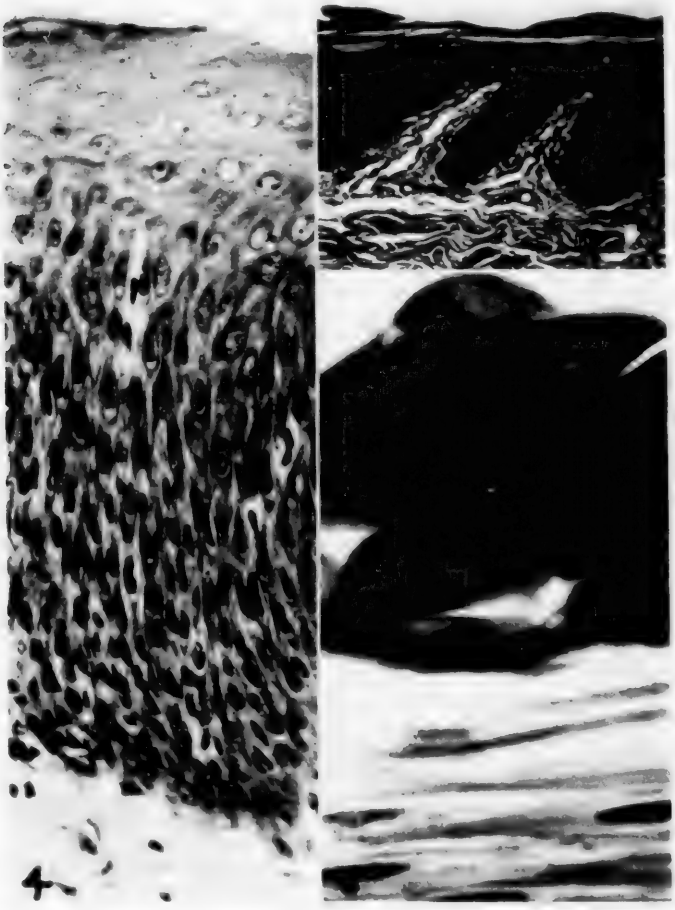


FIG. 4. MY ♯280 ♂ Castrated, 155 days. No other treatment. Shows chiefly castrate pattern. Note similarity to *fig. 2*. (Upper left ♯3 papilla alveolar gingiva—× 500).

FIG. 5. MY ♯303 ♂ Castrated, 150 days. No other treatment. Keratin and curls appear in areas where none is found normally; a change resembling that occurring in females treated with estrogenic hormone. (Upper central papilla areolar gingiva—× 170).

FIG. 6. Higher magnification of *fig. 5* showing details of keratin curls. (× 1360).

sectomized group as a whole than in the female castrate group, except in animal MY # 246 which more closely resembled the female castrates.

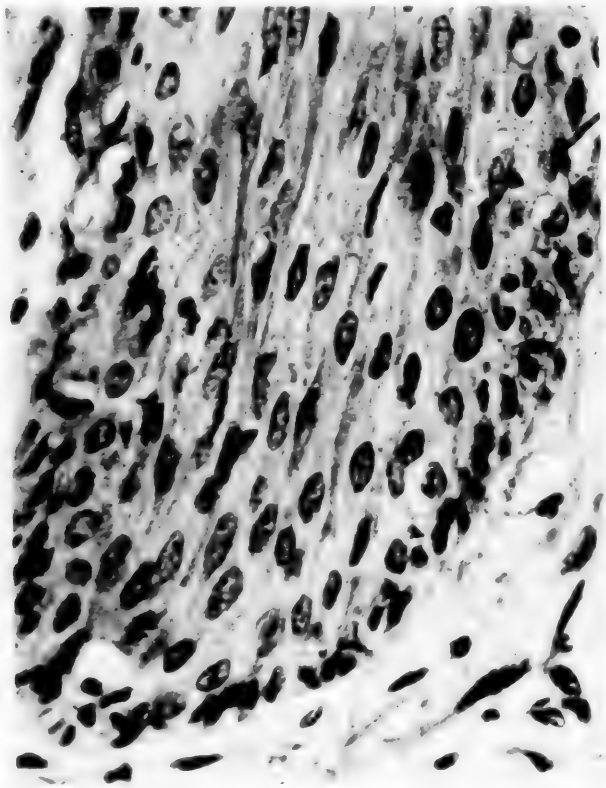


FIG. 7. MY #238 ♂. Hypophysectomized (complete removal) 47 days. No other treatment. Shows chiefly vertical arrangement similar to *figs. 2* and *4*. (Upper right #4 papilla alveolar gingiva × 650).

DISCUSSION

The interest in this study centers mainly about the effects of castration and hypophysectomy on the gingivae and oral mucous membranes of rhesus monkeys.

The so-called normal group showed some variation in histological

structure, as might be expected. The ages of the animals varied judging by hypothetical classifications determined from their weights. Thus, one weighing 1100 grams was regarded as a baby monkey;

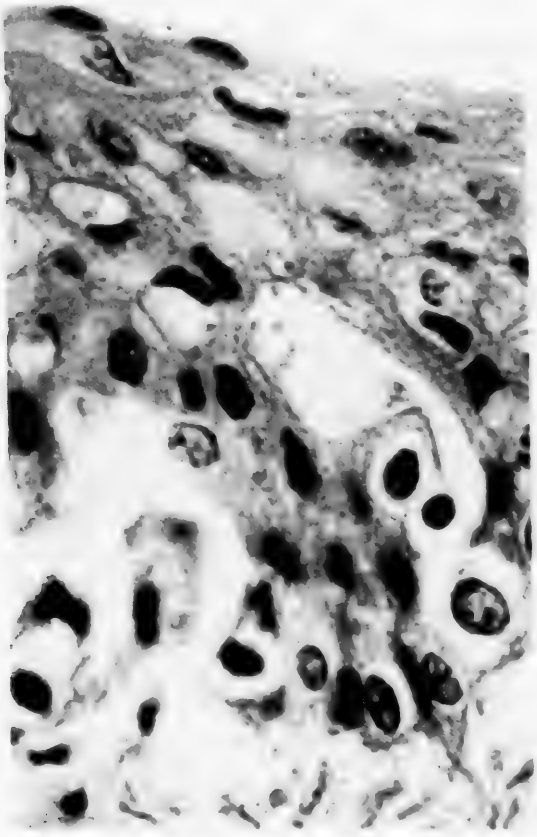


FIG. 8. MY *238 ♀ Same animal as in *figs. 1* and *7*. Shows marked tissue degeneration. Note similarity to *fig. 3*. (Upper right *4 papilla areolar gingiva ×1340).

others were either sub-adults or adults (Table I). Some of the animals had normal gingivae, some showed a mild gingivitis. The little-understood physiological state of the animals might have had some bearing on the structure of the gingivae. However, within these

limits, the gingival tissues of the normal series gave an uniform appearance under the microscope.

The impressive changes in the female castrate and hypophysectomized groups were: faint staining properties; altered arrangement of the prickle cell layer; and evidences of tissue degeneration, most apparent in the hypophysectomized monkeys. The stripping or fragmenting of the tissue was probably an artefact induced by the mechanics of preparation for the microscope because gross examination showed them to be whole and without ulcerations. This condition cannot be attributed to post mortem changes, as the stripping was observed also in sections taken a few days before some animals were sacrificed. However, it is to be noted that this type of artefact was not seen in any of the other groups.

Another factor of interest is the difference in response between the male and female castrates. While evidences of tissue degeneration were discernible in the latter, the other extreme, or hyperkeratinization, took place in the former. Should the hyperkeratinization described be due to effects of the estrogenic hormone (as seemed to be the case in previous studies) (1, 2), then the source of the estrogen is obscure.

CONCLUSION

Castration of female rhesus monkeys causes, chiefly, alteration of the arrangement of the prickle cell layer of the alveolar and areolar gingivae, and tissue degeneration.

Hypophysectomy causes marked degenerative changes in the gingivae and oral mucous membranes of female rhesus monkeys.

Castration of male rhesus monkeys causes changes in the gingivae and oral mucous membranes resembling the effects of injections with the estrogenic hormones; namely, hyperkeratinization.

We wish to acknowledge indebtedness and express our thanks to Doctors Earl T. Engle and Philip E. Smith for supplying most of the animals for this report.

REFERENCES TO LITERATURE

(1) Ziskin, D. E., Blackberg, S. N., Slanetz, C. A., *J. D. Res.* **15:** 407, 1936.
(2) Ziskin, D. E., *J. D. Res.* **16:** 367, 1937

THE EFFECT OF FLUORINE ON MOUTH BACTERIA[1]

B. G. BIBBY, B.D.S., PH.D., AND M. VAN KESTEREN, M.S.

Division of Dental Research, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

It has been suggested (1) that the inhibition of dental caries brought about by fluorides is the result of their action on the bacteria of the tooth surface. However, an attempt (2) to demonstrate that fluorides produced changes in the oral flora of rats was inconclusive. To cast light on the possible effects of fluorine on mouth organisms, a brief examination of the action of fluorides on bacteria is in order.

Flourine does not have marked bactericidal powers. The fluorine ion manifests variable activity according to the compound employed, and some organisms seem to be particularly resistant to its action (3).

There is some evidence that it is an effective fungicide (4). The ability of fluorine to inhibit the action of cellular enzymes has recently attracted much attention. The demonstration that low concentrations of fluorine interfere with the fermentation of carbohydrates (5) is of particular interest in relation to dental caries. Most of the studies on this inhibitory action have dealt with the fermentations in animal tissues or yeast, but there is no doubt that the phosphorolating mechanisms of bacterial fermentations are similarly affected (6). There are, however, few records of the effect of fluorine on bacterial enzymes. They consist of reports (7, 8) of limitation of metabolic activity at non-lethal concentrations, inhibition (9) of the oxidation of lactic and pyruvic acid at dilutions of NaF which did not affect respiration, and the finding (10) that somewhat different concentrations of NaF were required to prevent sucrose fermentation by single strains of colon bacilli and streptococci. It has also been shown (11, 12) that the breakdown of glucose and phosphoroglyceric acid, the key intermediate of the Embden-Myerhof fermentation chain, by propionic acid bacteria, does not take place in the presence of

[1] The work upon which this report is based was made possible by funds granted by the Carnegie Corporation of New York.

NaF, but that after subculture in fluoride-containing media these organisms are capable of fermenting glucose in fluoride concentrations which normally prevent the fermentation of phosphoroglyceric acid. This is taken to indicate that bacteria may have more than one mechanism for the dissimilation of carbohydrates.

While the action of fluorides in preventing the fermentation of carbohydrates suggests that such an effect may be concerned in the limitation of caries in man and animals ingesting fluorine, there is no direct evidence that this is the case, nor does it seem probable, considering the short period of time during which the fluorine-containing waters or solutions remain active in the mouth. In addition, there is no information regarding the effect of fluorine on the bacterial species which might be concerned in dental caries. Therefore, it seemed important to investigate the effect on the activity of oral bacteria of the fluorine concentrations likely to occur in the human mouth under conditions in which dental caries is being inhibited by fluorine-containing waters.

METHODS

Organisms: With the exception of three strains of lactobacilli isolated from milk, all the strains of organisms used were isolated from healthy human mouths just prior to, or during the course of the experiments. The oral lactobacilli were cultured from Hadley's tomato juice agar, the streptococci from oatmeal-bread agar or rabbit-blood agar. Blood agar was also used for the isolation of all other types except the fusiform bacilli which were obtained from potato-extract gentian-violet agar. Anaerobic conditions were needed for the isolation of the *Fusiformis* and *Leptotrichia*. Subcultures were maintained in Douglas' broth.

Media: A 5 per cent glucose Douglas' broth media was made by adding 1 volume of sterile 20 per cent glucose to 3 volumes of sterile Douglas' broth. Aliquots of 10 cc. were pippetted to 50 cc. Erlenmeyer flasks. When enamel or dentin was employed in the media, it was previously weighed and autoclaved in the flasks and the broth added after sterilization.

Fluoride Dilutions: Since marked reduction in dental caries is brought about by waters containing less than 2 parts per million of fluorine (13) and since mildly fluorosed teeth, containing approximately 250 and 400 p.p.m. of fluorine in enamel and dentin respectively (14), have a greatly enhanced resistance to caries, concentrations of fluorine between 1 and 400 p.p.m. should produce any of the effects on bacteria which might be responsible for the reduced activity of caries. Sodium fluoride contains 45.2 per cent of fluorine; therefore, NaF dilutions of from 2 to 1000 p.p.m. supplied the required fluorine concentrations. Sodium fluoride solutions containing 2, 10, 20, 50, 100, 250, and 1000 parts per 100,000 were prepared and sterilized. These were then diluted to ten volumes

by addition to the glucose Douglas' media giving final dilutions of 2, 10, 20, 50, 100, 250, and 1000 parts NaF per million, or from 0.9 to 452 p.p.m. of fluorine.

Two-hundred milligram amounts of fluorine-containing dental tissues were also used as a source of fluorine. For this purpose, incisor dentin from rats fed a high fluorine diet and "artificially fluorosed" dental tissues were utilized. The "artificially fluorosed" specimens consisted of enamel and dentin which as the result of a short treatment with a sodium fluoride solution had adsorbed sufficient fluorine to take on the property of lowered acid solubility, a characteristic of naturally fluorosed teeth (15).

TABLE I

Amounts of 0.1 N acid produced by oral streptococci in different NaF concentrations

	NaF conc. in p.p.m.							
	0	2	10	20	50	100	250	1000
8 Hours:								
No. of Tests	23	10	12	12	11	12	2	2
No. of Strains	8	5	6	6	6	6	2	2
Range (in ccs.)	1.34–3.39	1.97–3.07	1.68–3.23	1.54–3.14	1.31–2.72	1.10–2.53	0.43–1.06	0.45–0.46
Average (in ccs.)	2.31	2.48	2.49	2.48	2.13	1.89	0.75	0.46
Control Average (in ccs.)	—	2.43	2.66	2.66	2.66	2.66	2.78	2.78
Reduction	—	−0.05	0.17	0.18	0.53	0.77	2.03	2.32
24 Hours:								
No. of Tests	40	28	22	22	21	22	2	2
No. of Strains	14	13	9	9	9	9	2	2
Range (in ccs.)	1.98–4.34	1.98–3.42	1.61–3.98	1.58–3.75	1.31–2.90	1.19–2.56	0.81–1.28	0.39–0.60
Average (in ccs.)	3.20	2.71	2.62	2.46	2.00	1.81	1.05	0.50
Control Average (in ccs.)	—	3.08	3.26	3.26	3.26	3.26	3.73	3.73
Reduction	—	0.37	0.64	0.80	1.26	1.46	2.68	3.23

Procedure: Flasks were inoculated with 0.1 cc. amounts of 24 hour cultures of the test organisms and incubated at 37°C. Flasks containing enamel or dentin were rotated continuously. Tests were made at regular intervals, generally 8, 12, 24, 48, 72, and 96 hours after incubation, although in some experiments with the rapidly growing streptococci additional tests were made at intervals of 4, 6, 10, 16, and 20 hours. A separate flask was used for each test. Controls were supplied by incubating uninoculated media of each fluorine dilution for the duration of the experiment.

At the end of the incubation periods the pH of the media was determined by the use of nitrazine paper supplemented with spot tests with Brom-phenol-blue indicator in cases where pH was 4.5 or below. The amount of acid formed was determined by titration with 0.1 N NaOH using phenophthalein as the indicator and the first red color as the end-point. A burette calibrated to 0.01 cc. was used in this titration. When the flasks contained tooth substance, this was centrifuged out before the titrations were made.

FINDINGS

The effect of fluoride solutions on acid production: The 14 strains of streptococci tested produced acid most rapidly in the first 6 or 8 hours; thereafter acid formation was slower and virtually ceased after 24 hours. Since the values obtained at other time intervals conformed closely to an acid production curve of the type indicated, the effects of fluorine can be most simply presented by tabulating the results of the 8 and 24 hour titrations (Table I). It is noted that the amounts of acid formed are reduced proportionally with increasing concentrations of fluoride, until at concentrations of 1000 p.p.m. where no

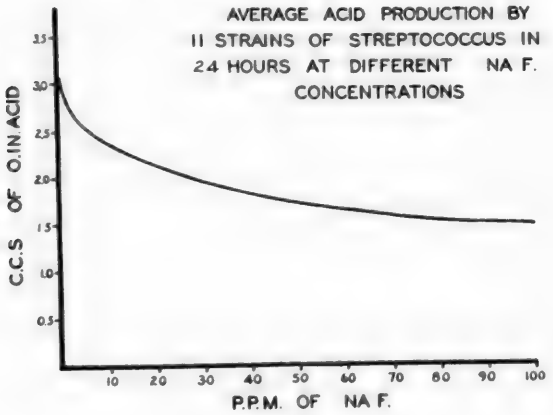


FIG. 1

definite growth occurred, the titratable acidities are no more than those of the uninoculated control flasks. A graphical representation of the effect of various fluoride concentrations on acid production by 11 strains of streptococci is given in *fig. 1*. *Fig. 2* shows the effect of different concentrations on the rate of acid formation by a single strain of streptococcus. *Fig. 3* presents typical results obtained in one of 10 experiments designed to show the relationship between fluorine concentration, acid formation and bacterial growth. For the sake of simplification, the 2, 10, and 50 p.p.m. tests, which gave consistent intermediate values, are omitted.

It is apparent from *fig. 3* that concentrations of fluoride up to 100

p.p.m. have no bactericidal effect and, further, that they do not interfere with bacterial multiplication to anything like the same degree that they inhibit acid production. Where no fluoride is present, acid elaboration apparently continues until its concentration becomes high enough to kill the organisms (a pH of from 4.0 to 4.5 reached at about 20 hours). Where the fluorides reduce acid formation, the bacterial population persists at a higher level. It is interesting to note that in the presence of fluoride, acid production continues only

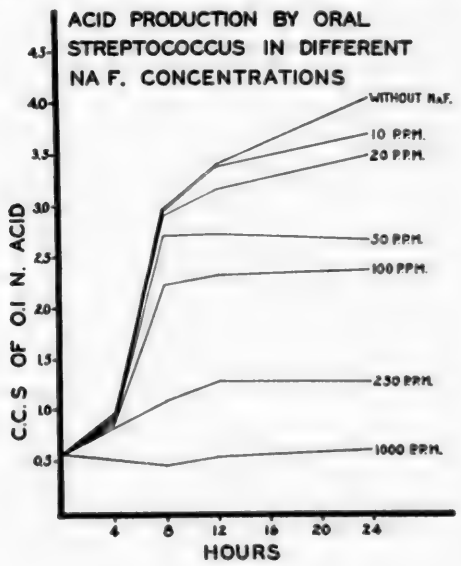


FIG. 2

as long as the bacteria are growing rapidly; whereas, in the control cultures, it continues apparently until the pH of the media becomes sufficiently low to inhibit enzyme action.

Two strains of streptococci, one a known active acid producer, the other a strain known to belong to the weak acid producing group, were subcultured and tested for acid production under anaerobic conditions. The amounts of acids formed in the various fluoride concentrations fell within the ranges given by the two organisms in previous aerobic tests.

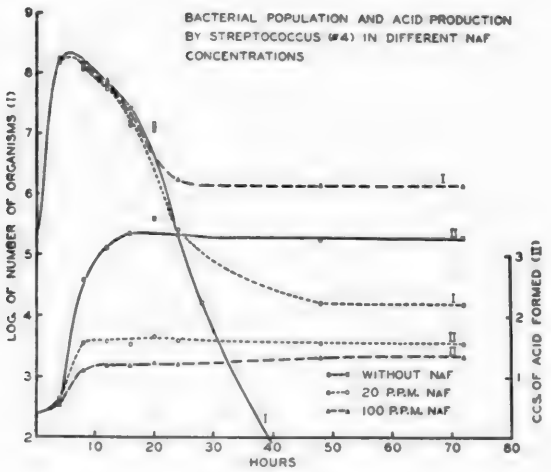


FIG. 3

TABLE II

Amounts of 0.1 N acid produced by lactobacilli in different NaF concentrations

	NaF conc. in p.p.m.							
	0	2	10	20	50	100	250	1000
24 Hours:								
No. of Tests	41	32	12	8	12	12	4	4
No. of Strains	12	10	6	4	6	6	4	4
Range (in ccs.)	0.90–3.03	0.77–2.99	0.97–3.03	1.01–3.01	0.98–3.00	0.81–2.86	1.33–1.49	0.98–1.16
Average (in ccs.)	1.97	1.81	2.04	1.93	1.87	1.64	1.41	1.07
Control Average (in ccs.)	—	2.63	1.96	1.92	1.88	1.88	2.08	2.08
Reduction	—	0.82	−0.08	−0.01	0.01	0.24	0.67	1.01
72 Hours:								
No. of Tests	41	32	12	8	12	12	4	4
No. of Strains	12	10	6	4	6	6	4	4
Range (in ccs.)	1.34–7.44	0.82–5.50	2.13–5.07	1.98–4.81	1.67–3.02	1.73–3.90	1.64–1.88	1.17–1.38
Average (in ccs.)	3.98	3.38	3.97	3.21	2.26	2.31	1.75	1.29
Control Average (in ccs.)	—	3.52	4.98	3.63	4.99	4.99	6.00	6.00
Reduction	—	0.14	1.01	0.42	2.73	2.68	4.25	4.71

The 12 strains of lactobacilli studied produced acid less rapidly than the streptococci, but under the conditions of the test continued to form it for much longer periods, giving final quantities of acid well in excess of the streptococci. The results of the 24 and 72 hour titrations, which show the important points on the acid production curves, are summarized in Table II. The effect of the additions of fluoride, especially the low concentrations, is not very marked at the 24 hour period, but at 72 hours the results of the additions of fluoride are much more noticeable. Concentrations of 1000 p.p.m. showed bactericidal effects.

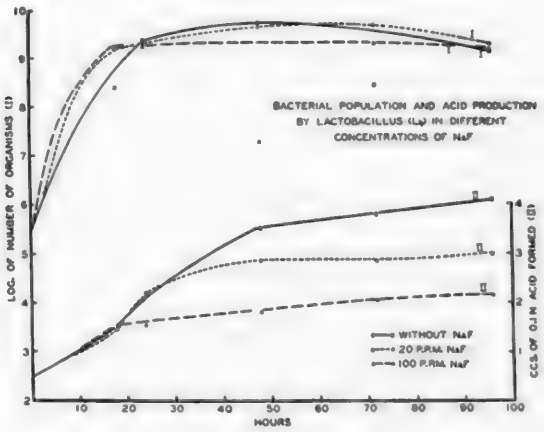


FIG. 4

In the 4 strains of lactobacilli tested there appeared the same type of interrelationship between growth and acid production as that shown for one strain in *fig. 4*. Here, as with the streptococci, there is an indication that in the presence of fluorine, acid production is most marked during the growth phase of the organisms but that thereafter it is very slight. It is apparent that fluoride concentrations up to 100 p.p.m. do not influence the growth of lactobacilli and, therefore, the effects on acid production must be the result of inhibition of a specific enzyme system of the micro-organisms.

The action of fluorine on acid production by 4 strains of micrococci, 3 Gram-negative cocci, 2 actinomyces, 2 fusiform bacilli, 2 leptotrichia

and 1 Gram-negative bacillus was also tested. None of these produced acid as rapidly as the streptococci, although after 24 hours, with the exception of the leptotrichia and the Gram-negative bacillus which gave absolutely no acid, all strains formed more than the lactobacilli. The reductions in the amounts of acid were proportional to the concentration of fluoride up to 1000 p.p.m. where growth was greatly limited. Although, because of the small number of strains

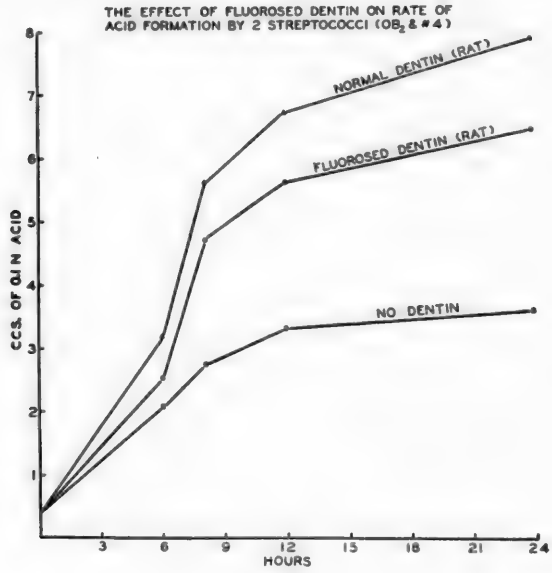


FIG. 5

tested, it cannot be regarded as significant, the acid production by the fusiform bacilli seemed somewhat less affected than that of the other types.

The effect of fluorine-containing enamel and dentin on acid formation: Incorporation of enamel and dentin in cultures of organisms either increased or reduced the amount of acid according to the bacterial type. Regardless of the reaction of the particular strain, however, the amount of acid produced by cultures of streptococci was reduced by as much as 20 per cent by additions of heavily fluorosed incisor dentin from rats fed a high fluorine diet. The rate of acid production for 2

tests is shown in *fig. 5*. Dentin from animals fed a low fluorine diet (20 p.p.m.) did not influence acid formation differently from normal rat dentin.

A reduction in acid production was also brought about by the addition to cultures of "artificially fluorosed" enamel and dentin. The results of tests of this type are shown in *fig. 6*. The 24 hour tooth substance tests with different organisms are summarized in *fig. 7*.

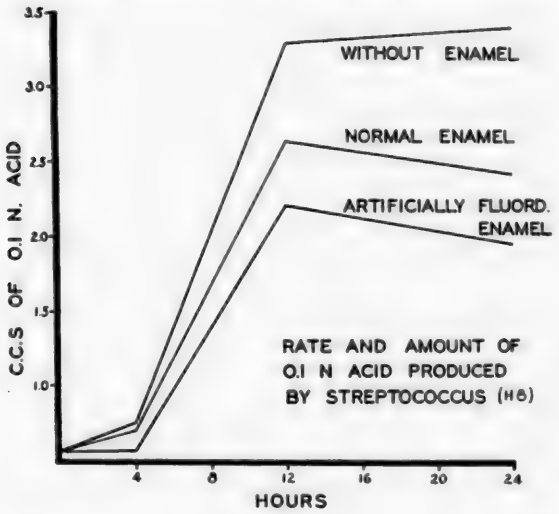


FIG. 6

The contrasted results produced by different organisms in the presence of different tooth substances will be discussed in another report.

Counts of the bacterial population carried out in conjunction with the above tests showed that the fluorosed dental tissues had no effect on the growth of the organisms other than that brought about by the buffering action of the enamel or dentin. The result of this was that considerable numbers of the more aciduric strains of organisms were still viable at the end of the experiments. The population peak was, however, the same, and was reached at the same time whether enamel or dentin were present or not. Differences between the acid production in the fluorine-containing and fluorine-free dental tissues did not appear until after the bacteria had ceased to multiply rapidly.

DISCUSSION

The results show that fluorine concentrations likely to be encountered in the mouth will not interfere with bacterial growth but that they are capable of preventing the formation of acids from carbohydrates. The extent of interference with fermentation is proportional to the concentration of fluorine. But since fluorine concentrations of no more than one part in a million show a slight effect, it is

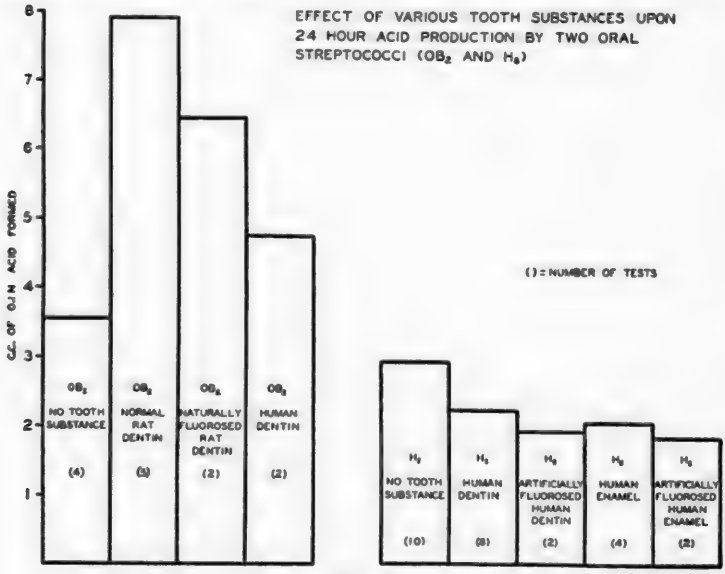


FIG. 7

apparent that the water from fluorine-containing supplies would have some influence on acid formation. However, the effects of such waters would be acting for only a few minutes each day and consequently it does not seem likely that their action would significantly alter acid production in the mouth. The finding that fluorosed dentin exerts a marked inhibitory action seems to have more importance because the fluorosed dental tissues would exert a continuous effect in the region of the tooth surface, the site where acid production is most important in initiating dental caries. Unfortunately, the fluorine

content of the teeth tested was much greater than has been encountered in human teeth and so the reasoning does not necessarily apply to the human mouth. The demonstration that human enamel and dentin adsorb sufficient fluorine to reduce bacterial acid production in their immediate environment is of great interest and suggests that fluorine from drinking water may be held on the tooth and thereby prevent acid formation in the region of the tooth surface long after the fluorine-containing water has passed from the mouth.

Our correlated studies of the effect of fluorine on bacterial growth and acid production are the first of this sort to be reported. The fact that in all these tests, even when tooth substance buffers were present, the inhibitory effect of fluorine was much less during the active growing phase of the organisms than when they had ceased to multiply suggests either that the fluorine is less effective on the enzymes of multiplying bacteria, or that during that phase the organisms have an additional fermentation mechanism which is not affected by fluorine.

SUMMARY

The effects of various concentrations of sodium fluoride solutions on the growth and acid production of streptococci, lactobacilli and other mouth organisms were tested. The action of fluorosed, and fluorine-treated dental tissues were similarly studied.

Fluorine concentrations of less than 1 p.p.m. limit acid production by bacteria but concentrations in excess of 250 p.p.m. are needed to affect bacterial growth.

Fluorosed and fluorine-treated enamel and dentin also reduce acid production by bacterial cultures.

REFERENCES TO LITERATURE

(1) Miller, Ben J., *Proc. Soc. Exp. Biol. and Med.*, **39**: 389, 1938.
(2) Harrison, R. W., *Proc. Soc. Exp. Biol. and Med.*, **39**: 459, 1938.
(3) Eisenberg, P., *Cent. f. Bakt., Ab. I, Orig.*, **82**: 69, 1919.
(4) Moll, F., *Cent. f. Bakt., Ab. II, Orig.*, **51**: 257, 1920.
(5) Lipmann, F., *Biochem. Zeitschr.*, **196**: 3, 1928.
(6) Werkman, C. H., *Bacteriological Reviews*, **3**: 187, 1939.
(7) Lockemann, G., and Lucius, F., *Desinfektion*, **5**: 261, 1913.
(8) Meyerhoff, O., *Arch. Physiol. (Pfluger)*, **166**: 240, 1916.
(9) Barron, E. S. G., *J. Biol. Chem.*, **113**: 695, 1936.

(10) WRIGHT, H. D., *J. Path. and Bact.*, **45**: 117, 1937.
(11) WERKMAN, C. H., STONE, R. W., WOOD, H. G., *Enzymologia*, **4**: 24, 1937.
(12) WIGGERT, W. P., WERKMAN, C. H., *Biochem. Journ.*, **33**: 1061, 1939.
(13) DEAN, H. T., *et al*, *Public Health Reports*, **54**: 862, 1939.
(14) ARMSTRONG, W. D., *J. D. Res.*, **17**: 393, 1938.
(15) VOLKER, J. F., *Proc. Soc. Exp. Biol. and Med.*, **42**: 725, 1939.

A WROUGHT METAL FOR TECHNIC USE

GEORGE S. EASTON, D.D.S., M.S.

College of Dentistry, State University of Iowa, Iowa City, Ia.

The high cost of wrought gold alloys prohibits their use as technic material and necessitates the employment of some suitable material in practice manipulation and the construction of demonstration models. This substitute material should possess physical and metallurgical properties quite similar to those possessed by the approved wrought gold alloys. In addition thereto it should be relatively low in cost.

The important requirements for a satisfactory wrought technic metal may be stated as follows: 1. It should "plier" readily and possess an adaptability and formability similar to that of the wrought gold alloys. 2. The lower limit of the melting range should be high enough to permit a safe soldering procedure. 3. It should be capable of being soldered without the use of a special technic. 4. It should be capable of taking a high polish and should remain reasonably resistant to atmospheric corrosion. 5. It should possess a satisfactory color. 6. It should be responsive to a heat treatment similar to that employed for the wrought gold alloys.

An inexpensive wrought metal which meets these requirements would prove to be valuable in the construction of display models and in practice manipulation of wrought materials. This study was instituted for the purpose of investigating several possible sources of such material and, if possible, to find one that would satisfactorily serve its intended purpose.

The copper-zinc and copper-tin alloys (brasses and bronzes) were the first to be considered as a possible source of a satisfactory wrought technic material. It was found that the brasses and bronzes having satisfactory hardness, strength and resiliency are not manufactured into wire because of their low malleability and ductility but are used only for casting. Those that are available as wires have a low elastic limit and tensile strength, especially in the softened condition. Fur-

thermore, these alloys can not be hardened by any known method of heat treatment.

A casual study of scientific and trade literature pertaining to likely metals and alloys revealed 3 possible sources of the desired material: 1. An alloy of nickel containing copper and silicon; 2. an alloy of copper and beryllium; and 3. the complex stainless steel alloys, such as one of the nickel-chromium group containing beryllium. Further investigation indicated that the stainless alloys and the alloys of nickel with copper and silicon would probably fail to yield the desired results. It is possible that a nickel-copper-silicon alloy might be developed which would possess the necessary physical properties. It would not, however, be satisfactory from the standpoint of heat treatment. While it is true that these alloys respond to a type of heat treatment in use with the wrought golds, the required temperatures are beyond the range of practicability. The softening treatment would require a temperature of about 1900° F. while the ageing or hardening treatment would have to be done at a temperature of from 1000° F. to 1200° F.

The complex stainless alloys were discarded for two reasons. The nickel-chromium-iron alloys containing beryllium that respond to heat treatment are not being manufactured into wire. The alloys of this group can not be readily soldered by the more common soldering technics.

The remarkable properties of copper-beryllium alloys have been known since 1929.[1] The alloys of this system containing from 2 to 2½ per cent beryllium have played an important role in the fabrication of articles requiring a copper-base alloy having high formability, high corrosion resistance and good response to heat treatment. Since these characteristics constitute three of the more important requirements of the material under consideration it was decided to study the properties of an alloy of copper and beryllium containing about 97 per cent copper and up to 3 per cent beryllium.[2] For simplicity this alloy shall hereinafter be known as B-C metal.

[1] *Beryllium-Arbeiten*, Siemens-Konzern (Berlin: Julius Springer, 1929).

[2] The author is indebted to General Refineries, Inc., from whom this alloy was obtained, and to K. W. Ray, Director of Research of General Refineries, for his analyses of the material and assistance in preparing test wires.

Test samples were prepared from ingots containing 97.2 per cent copper and 2.8 per cent beryllium. Since beryllium is readily lost by oxidation during softening heat treatments, a later analysis was made on discarded test samples of 18 gauge (0.040) wires. In this instance the beryllium content was 2.2 per cent. Hence the probable beryllium content of test samples used in this study was 2.0–2.5 per cent. The melting range of the 2.5 per cent beryllium wire is 1587°F.–1751°F. (864°C.–955°C.). The fusion temperature, by the wire method, is 1702°F. (938°C.).

The color of B-C metal in its natural state is predominately that of copper. In the polished state it presents an appearance comparable to that of many of the hard golds. The objectionable "reddish" tinge so frequently observed in alloys of high copper content is not present.

Since it is a base metal alloy, high in copper, B-C metal is not entirely resistant to atmospheric corrosion, and is not at all satisfactory for use in the mouth. It is capable of being highly polished. Once it has been properly finished and polished but little effort is required to maintain a bright appearance. It can be plated by the use of standard plating procedures. Gold plating is readily accomplished and cases so treated present a most pleasing appearance.

Free hand and investment soldering operations were performed to determine the solderability of B-C metal. Both silver technic solder and gold solder of .650 fineness were used. Some difficulty was encountered when free hand soldering was attempted with technic solder and borax flux. This situation was relieved by the use of a fluoride flux in ample amounts. No trouble was encountered when a .650 fine gold solder was used.

B-C metal can not be used for casting because of the formation of beryllium oxide upon the surface of the molten alloy. The beryllium oxide forms a tough coating which flux will not remove.

The effect of heat treatment upon this material was determined upon cold rolled samples approximately 10 mm. square and 2 mm. in thickness and upon 18 gauge (0.040) wires. The cold rolled samples presented a Rockwell "C" hardness of 28. All samples were treated in an automatically controlled electric furnace which had been preheated to the desired temperature. Upon removal from the furnace

the samples were immediately quenched in water at room temperature. The effect of heat treatment was determined by hardness and stiffness tests. For hardness determinations the Rockwell hardness tester was used, the "B" scale being used for soft samples and the "C" scale for those that had been hardened. The Tinius Olsen stiffness

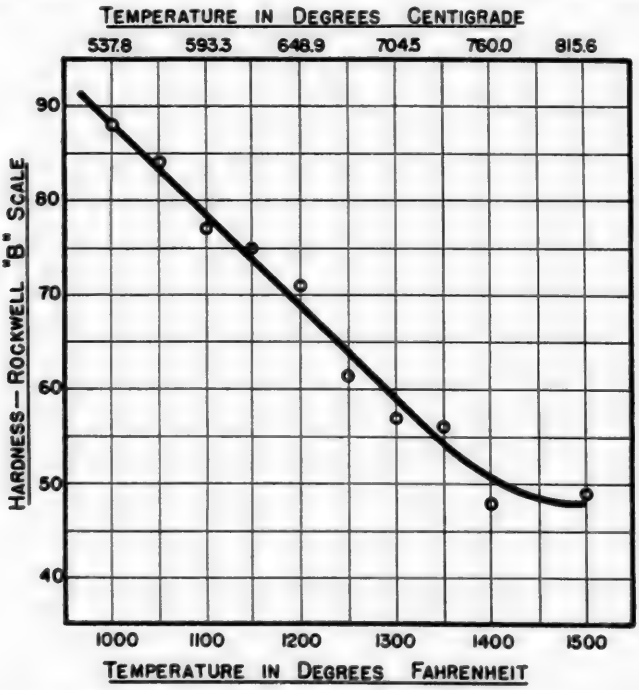


FIG. 1. The effects of softening heat treatments on the hardness of B-C metal. (Samples were heated at stated temperatures for 10 minutes and then quenched in water at room temperature.)

tester was used for stiffness determinations. Test values were obtained by using the average of the results of three tests upon each sample.

The effect of a softening treatment upon B-C metal was studied upon samples which had been treated for ten minutes at temperatures varying from 1000°F. (538°C.) to 1500°F. (816°C.) and quenched. *Fig. 1* shows the results of these treatments. The hardness of the

material decreased from a Rockwell "B" value of 104 (obtained by conversion from "C" 28) in the cold rolled condition to 48 when treated at 1400°F. (760°C.).

In order to determine the effect of time as a factor in the softening treatment, samples were heated at 1300°F. (704.5°C.) and 1350°F. (735°C.) for periods of time varying from five to thirty minutes. The samples heated at 1300°F. (704.5°C.) presented maximum softness at the end of twenty minutes, with but slight changes in hardness

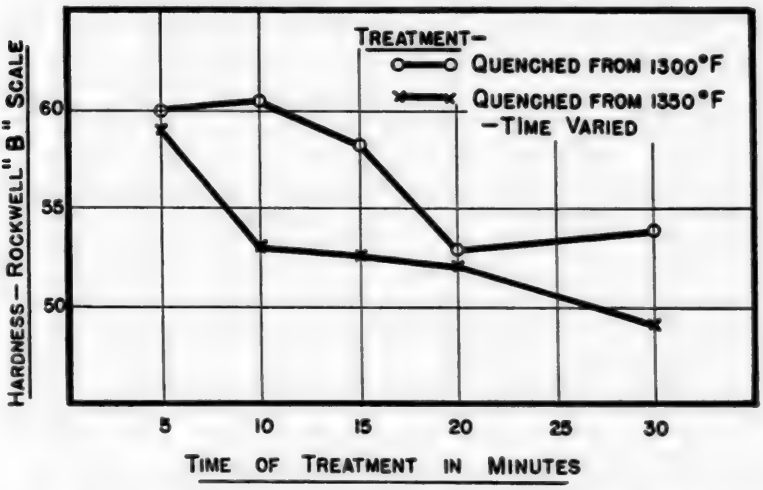


FIG. 2. The effects of softening B-C metal at 1300°F. and 1350°F. for varying periods of time.

occurring when the treatment time was prolonged. When the temperature was increased to 1350°F. (733°C.) ten minutes of treatment produced the same degree of softness as did twenty minutes at 1300°F. (704.5°C.). Maximum softness occurred with the thirty-minutes treatment. Results of these treatments are shown in *fig. 2*. The 1350°F.–10 minutes treatment was selected as the softening treatment to be used throughout this study. All samples were so treated and quenched in water at room temperature before being subjected to a hardening treatment.

Since thirty minutes is the time period frequently used in the harden-

ing treatment of wrought golds, it was selected as the initial time factor to be used in determining the hardening properties of B-C metal. Previously softened samples were heated for thirty minutes at temperatures ranging from 400°F. (204°C.) to 700°F. (371°C.) and varying by intervals of 50°F. (28°C.). A definite hardening effect occurred at 450°F. (232°C.), the maximum being reached at 550°F. (288°C.). The changes in hardness, obtained by using the Rockwell "C" scale, are shown in *fig 3*. For the purpose of comparison with hardness val-

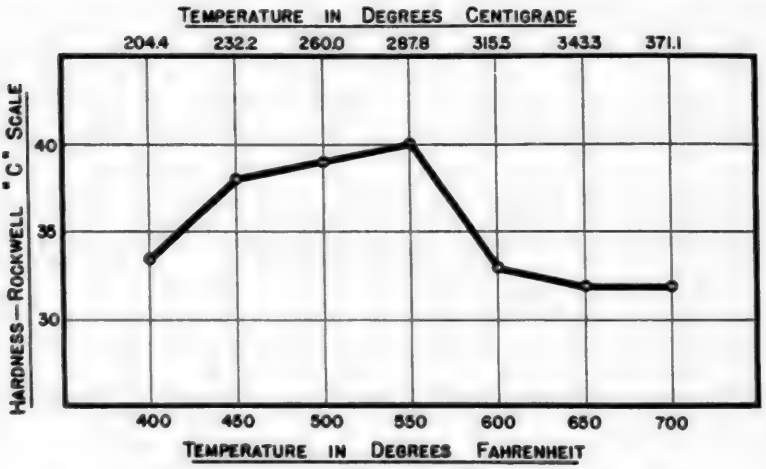


FIG. 3. The effects of hardening heat treatments on the hardness of B-C metal

ues shown in *figs. 1* and *2*, Rockwell "B" values are shown on the right side of the graph. These values were obtained by conversion and are only approximate.[3]

The effect of the time factor in the hardening treatment was determined upon samples heated at 500°F. (260°C.) and 550°F. (288°C.) for 5, 10, 15, 20 and 30 minutes (*fig. 4*). In both instances the hardness increased with an increase in treatment time. Maximum hardness was closely approached following a twenty-minutes treatment, and there was but a slight difference between values for the samples heated at 500°F. and those heated at 550°F.

[3] Metals Handbook, (Cleveland, American Society for Metals: 1936), p. 40.

In order to check the response of B-C metal to other modes of hardening, previously softened samples were hardened by oven-cooling and by a salt bath treatment. The Dee Heatreat unit was used for the latter treatment. Test samples were submerged for twenty minutes in the molten salt, the temperature of which remained reasonably constant at 500°F. (260°C.). Samples so treated presented a Rockwell "C" hardness of 36.

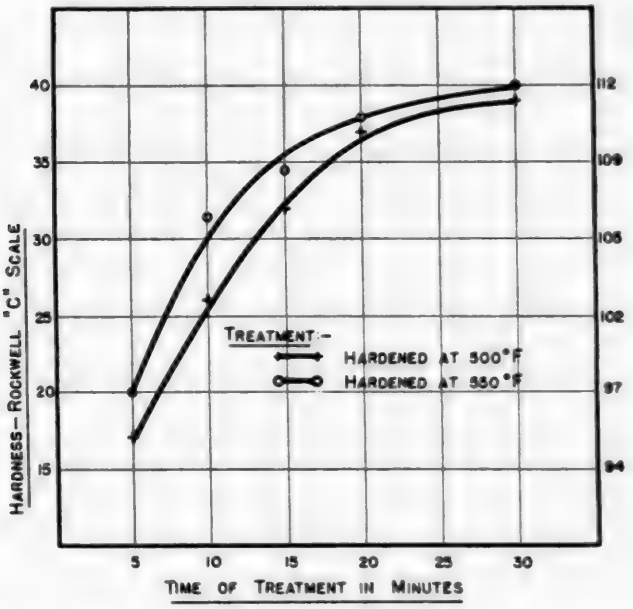


FIG. 4. The effects of hardening B-C metal at 500 F. and 550 F. for varying periods of time.

A Woodbury-Jelenko furnace was used for the oven-cooling treatment. Samples were placed in the furnace at 840°F. (449°C.) held at that temperature for two minutes, and then cooled from 840°F. to 480°F. (449°C.-249°C.) in fifteen minutes. This treatment produced a Rockwell "C" hardness of 22.

The plastic properties of B-C metal following various conditions of heat treatment were studied by means of the Tinius Olsen stiffness tester. This machine bends a wire to a 90° angle by a continuous and

automatically applied load. Both load and angle of bend may be read continuously. Tests were made upon 18 gauge (0.040) wires,

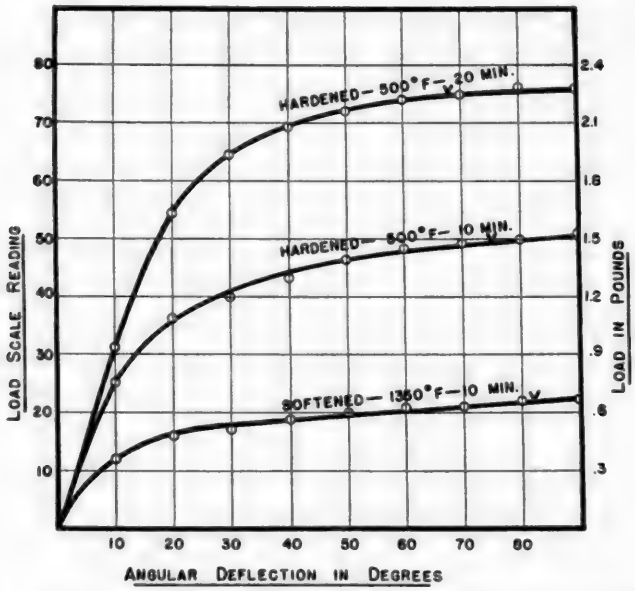


FIG. 5. A comparison of the bending properties of B-C metal following various methods of heat treatment.

TABLE I

A comparison of the properties of B-C metal and a 6% pt. wire

		BRINELL HARDNESS	FUSION TEMP.	TENSILE STRENGTH	ELASTIC LIMIT	ELONGATION, % IN 2 IN.
B-C Metal	S*	112	1702 F.	67,000	28,000	32
	H*	350		148,000	116,500	4
6% Pt. Wire	S	152	1706 F.	96,000	58,000	22
	H	248		133,000	104,000	5

* S.—Softened for 10 minutes at 1350°F. H.—Hardened at 500°F. for 20 minutes.

using a bending moment of 3 inch-pounds and a span of 1 inch. Comparative results are shown in *fig. 5*.

The 90° bending strength or stiffness of a 1 inch span of wire

hardened at 550°F. (260°C.) for twenty minutes was approximately 32.5 per cent greater than that hardened at the same temperature for ten minutes, and about 235 per cent greater than the 1–90 stiffness of the softened samples. The angle of set (shown by the "V" near the top of each curve), which indicates the amount the wire has been permanently bent when the load is removed, was only about 95 per cent as great for the wires hardened at 500°F. for twenty minutes as it was for the softened samples. The angle of elastic flexure (90° minus the angle of set) was greater for the hardened samples. This angle of elastic flexure was about 275 per cent greater for the 500°F.–20 minutes hardened wires than for the softened wires.

Tensile tests were made upon 18 gauge (0.040) wires in both the softened and hardened condition. Values for tensile strength, elastic limit and elongation of B-C metal are compared with those of a popular 6 per cent platinum wire in Table I.

CONCLUSIONS

Possible sources of a satisfactory wrought technic metal have been investigated. An alloy of copper and beryllium containing 2–2½ per cent beryllium, herein termed B-C metal, has been studied to some extent. Results of this study indicate that the properties of B-C metal are such that it may well serve as a technic substitute for wrought gold alloys in practise manipulation and the construction of display models. In strength, elasticity, elongation and melting range it is quite comparable with many of the wrought golds. It will receive and retain a high polish, in which state its color is satisfactory. It gold plates readily. It responds to the same type of heat treatment procedures as do the wrought golds, and its response is such that its properties can be varied to a large extent. It can be soldered with either technic or gold solder, although the operation is not accomplished as easily as it is when wrought golds are used. B-C metal can not be used repeatedly because of the loss of beryllium by oxidation at high temperatures and consequent softness. It can not be cast satisfactorily.

CHEMICAL ANALYSES OF DECIDUOUS ENAMEL AND DENTIN[1, 2]

M. J. BIRD, B.A., ETHEL L. FRENCH, PH.D., MARIAN R. WOODSIDE, B.A., MARTHA I. MORRISON, AND HAROLD C. HODGE, PH.D.

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, and the Department of Chemistry, College of Arts and Science, the University of Rochester, Rochester, N. Y.

INTRODUCTION

Although a few analyses of deciduous teeth have been reported (1, 2, 3, 4) the information concerning their composition is incomplete. The previous workers made no attempt to determine average values and normal limits of variation for even the more important constituents.

In the present analyses, the separated enamel and dentin of deciduous teeth were analyzed for: 1. the relative amounts of moisture and organic and inorganic matter, 2. the calcium and phosphorus percentages of the inorganic residue. Averages and normal limits of variation were computed for the analytical values. These tissues possessed the same "remarkable constancy of composition" previously reported for fetal (5) and adult (6, 7, 8) teeth. In fact the similarities in chemical composition of deciduous and adult enamel and dentin are more impressive than the minor differences.

METHOD

The deciduous teeth were obtained from the Rochester Dental Dispensary through the courtesy of Dr. H. J. Burkhart and Dr. E. J. Pammeter. After the teeth were cleaned and carious material removed, enamel and dentin were separated by the Manly and Hodge flotation process (9). All the enamel and all the dentin from each tooth were used for analysis. The percentage of moisture was determined by the method given by LeFevre and Manly (6). The organic matter was extracted with potassium hydroxide in ethylene glycol (10). Calcium determinations were made according to the Sobel and Sklersky

[1] This work was supported in part by the Carnegie Corporation of New York.

[2] Read at the 18th General Meeting of The International Association for Dental Research, Philadelphia, March 13, 1940 (*J. D. Res.*, 19: 288, 1940) and at the Cincinnati Meeting of the American Chemical Society, April 9, 1940.

TABLE I

Classification of teeth

TOOTH NO.	LOCATION[1]	CARIES[2] CLASS	SEX[3]	AGE	GENERAL[3] HEALTH	ORAL[3] HEALTH	REASONS FOR EXTRACTION
402	⌊III	II	F	11	F	P	Orthodontic
403	⌊II	0	F	6	G	G	Orthodontic
404	⌊III	II	F	11	F	P	Orthodontic
405	I⌋	I	M	6	G	F	Decay
406	I⌋	II	M	7	F	F	Decay exposed pulp
407	⌊IV	II	F	5	F	F	Abscessed
408	V⌋	II	M	8	F	F	Abscessed
409	II⌋	0	M	7	F	F	To make room for permanent tooth
410	⌊IV	I	F	6	F	F	Fistula-beginning abscess
411	IV⌋	I	M	12	F	F	Fistula and abscess
412	⌊V	II	M	7	F	F	Decay
413	⌊IV	III	M	8	F	F	Decay
414	V⌋	I	M	9	F	F	Exposed pulp and loose
415	⌊III	I	M	10	F	F	Loose
416	IV⌋	II	M	10	F	F	Decay
417	⌊V	II	M	12	F	F	Decay
418	V⌋	I	M	11	F	F	Decay
419	IV⌋	I	F	5	F	F	Decay
420	V⌋	I	F	6	F	F	Loose—decay
421	⌊IV	I	M	6	F	F	Abscess
422	⌊V	I	M	6	F	F	Decay—exposed pulp
423	V⌋	III	M	8	F	F	Decay
424	V⌋	II	M	8	F	F	Decay
425	⌊IV	III	F	5	F	F	Orthodontic—cleft palate
426	⌊IV	II	M	10	F	F	Decay—loose
427	IV⌋	II	M	12	F	F	Decay
428	IV⌋	III	M	5	F	F	Decay—exposed pulp
429	V⌋	II	M	6	F	F	Orthodontic—fistula (abscess)
430	⌊V	I	M	9	F	F	Decay—fistula
431	⌊V	II	M	10	F	F	Decay
432	V⌋	III	M	5	F	F	Decay—exposed pulp
433	⌊IV	I	F	8	F	F	Abscess
434	V⌋	II	M	6	F	F	Carious
435	⌊V	I	F	6	F	F	Fistula
436	IV⌋	I	M	8	F	F	Carious
437	V⌋	II	M	8	F	F	Carious
438	IV⌋	I	F	8	F	F	Fistula
439	⌊V	I	F	9	F	F	Carious
440	⌊IV	II	M	5	F	F	Carious
441	⌊V	II	M	5	F	F	Carious
442	IV⌋	I	F	7	F	F	Carious
443	⌊IV	II	M	8	F	F	Carious
444	IV⌋	II	M	8	F	F	Carious
445	V⌋	I	F	7	F	F	Carious
446	V⌋	I	M	5	F	F	Buccal fistula

[1] The notation for location is as follows: I first incisor, II second incisor, III canine, IV first deciduous molar, V second deciduous molar, ⌊ indicates upper right, ⌋ upper left, ⌈ lower right, ⌉ lower left.

[2] Caries classes range from 0 with no caries to IV with severe caries.

[3] M male, F female, G good, F fair, P poor.

TABLE II

Analytical values for the enamel and dentin of deciduous teeth

TOOTH NO.	MOISTURE[1] (% MOIST WT.)		ORGANIC (% DRY WT.)		CALCIUM (% INORG. WT.)		PHOSPHORUS (% INORG. WT.)		Ca/P	
	E	D	E	D	E	D	E	D	E	D
402	6.6	*0.4*	*34.3*	19.8	36.5	34.6	18.8	16.8	1.94	2.06
403	5.9	12.3	2.5	11.3	*25.4*	29.6	*12.0*	14.6	2.11	2.02
404	5.9	4.2	11.9	16.0	36.1	20.7	17.2	16.4*	2.10	*1.24*
405	*0.0*[2]	8.2	*19.1*	23.2	31.2	*16.9*	14.8	*8.7*	2.10	1.94
406	1.8	11.2	*55.6*	42.0	*25.6*	35.2	*12.1*	16.3	1.98	2.16
407	1.5	10.3	*17.8*	16.2	37.5	29.2	17.0	13.7	2.20	2.13
408	1.6	9.0	1.2	25.0	37.3	23.5	17.9	11.3	2.09	2.08
409	1.7	9.9	8.3	22.5	34.8	30.8	15.8	14.6	2.20	2.12
410	1.8	9.3	4.8	27.4	37.2	35.1	18.3	16.6	2.03	2.11
411	1.6	9.8	7.3	28.4	37.4	27.9	18.4	13.0	2.03	2.15
412	3.6	12.9	4.5	19.1	35.4	37.0	17.2	16.8	2.05	2.20
413	2.4	12.8	1.9	21.3	38.6	36.6	18.0	16.9	2.14	2.17
414	2.3	13.2	3.0	24.0	37.8	36.6	18.1	16.9	2.09	2.16
415	3.8	12.7	6.2	19.4	36.1	33.7	16.9	17.0*	2.14	1.99
416	2.9	7.1	6.6	31.8	39.9	34.3	18.7	16.0	2.13	2.15
417	*0.1*	10.0	2.7	31.8	32.2	35.9	16.5	16.7	1.99	2.15
418	2.2	*51.1*	9.0	29.8	37.4	37.6	17.3	17.6	2.16	2.14
419	2.1	11.0	7.8	21.0	35.2	33.9	17.5	16.5	2.01	2.06
420	2.8	11.6	1.5	22.0	37.3	38.8	18.0	18.0	2.08	2.16
421	2.5	12.2	3.9	22.1	39.0	39.0	18.0	17.8*	2.16	2.19
422	2.3	10.4	4.3	5.1	39.9	28.0	18.5	14.4	2.14	1.95
423	1.6	11.8	5.6	40.4	*25.9*	37.5	17.2	17.3	*1.50*	2.17
424	2.0	9.0	5.5	26.8	38.3	46.2	19.1	21.9	2.00	2.12
425	4.5	12.9	3.1	23.3	40.7	33.6	21.4	16.8	1.91	2.10
426	4.6	13.4	7.5	36.4	38.9	31.1	18.3	15.4	2.13	2.01
427	3.5	13.4	12.1	22.4	36.2	36.3	17.2	16.6	2.10	2.19
428	3.3	11.9	7.3	31.5	37.6	*15.9*	19.0	*7.3*	1.98	2.18
429	3.2	13.2	6.3	15.9	37.5	37.6	17.8	17.3	2.11	2.18
430	3.0	10.3	2.5	32.6	41.1	36.6	18.3	16.6	2.24	2.20
431	2.1	10.6	2.7	13.7	39.6	34.3	18.2	16.5	2.17	2.08
432	2.8	11.8	2.1	23.5	37.7	34.8	18.3	18.5	2.06	1.88
433	*0.2*	*21.8*	*73.4*	*61.9*	36.6	20.2	17.2	10.6	2.13	1.90
434	2.2	10.8	12.2	31.4	37.4	36.4	18.2	18.8	2.05	1.94
435	1.7	16.7	*46.2*	lost	36.8	lost	18.8	lost	1.96	lost
436	0.5	12.4	*74.8*	19.9	35.4	35.5	18.3	17.2	1.94	2.06
437	4.1	12.7	1.4	23.7	34.7	34.5	14.5	16.8	*2.39*	2.05
438	3.1	11.2	4.4	23.7	35.1	35.6	18.0	17.5	1.95	2.04
439	*0.2*	9.1	3.0	20.3	36.8	34.9	18.6	17.4	1.98	2.01
440	*0.1*	*1.1*	3.6	26.5	37.2	35.8	17.9	17.3	2.08	2.07
441	*0.1*	*1.2*	0.7	26.1	35.3	35.7	17.0	17.5	2.08	2.04
442	*0.1*	*1.3*	2.7	11.2	36.3	28.8	17.8	14.5	2.04	1.99
443	0.8	*0.8*	1.8	30.3	35.9	34.2	18.9	17.2	1.91	1.98
444	*0.1*	*0.7*	3.5	27.4	35.2	35.1	lost	16.9	lost	2.08
445	*0.2*	*2.4*	7.9	20.7	36.8	37.2	18.1	17.6	2.09	2.11
446	*0.0*	*2.2*	2.0	23.5	33.8	35.6	18.1	18.1	1.87	1.96

* All values given for calcium and phosphorus are the average of duplicate determinations except those marked with an asterisk.

[1] The term "moisture" is here used advisedly in preference to "water," since it is recognized that part of the water content of the tooth substance is held as "water of crystallization" or "water of constitution" and is not driven off at 100° C. (5).

[2] Numbers which are italicized are discarded when calculating the averages.

micromethod (11) by precipitating as calcium oxalate, converting (at 475–525°C) it to calcium carbonate. This was dissolved in boric acid and titrated against hydrochloric acid to the pH of pure boric acid. Phosphorus was determined by the Holtz (12) micromethod in which the phosphorus was converted to and weighed as ammonium phosphomolybdate.

DATA

Table I tabulates descriptive material for the 45 deciduous teeth used in these analyses. The teeth were extracted from white children between the ages of 5 and 12. Except for 4 incisors and 3 canines, all the teeth were molars; and except for 2 incisors, all the teeth showed caries.

TABLE III

Statistical summary of data in table II

	MOISTURE (% MOIST WT.)		ORGANIC (% DRY WT.)		Ca (% INORG. WT.)		P (% INORG. WT.)		Ca/P	
	E	D	E	D	E	D	E	D	E	D
Average of all data.....	2.2	10.5	11.2	24.8	36.1	33.1	17.6	16.1	2.06	2.06
Average (corrected)[1].....	2.8	11.1	4.9	24.0	36.0	33.3	17.8	16.5	2.06	2.08
Standard deviation (corrected).....	1.6	5.6	3.1	7.3	2.0	4.6	1.2	2.0	0.09	0.09

[1] The "corrected average" is calculated after discarding the numbers italicized in Table II. The "standard deviation corrected" is calculated from the numbers used in obtaining the "corrected average."

Table II presents the results of the chemical analyses of the deciduous teeth as: the percentages of moisture expressed as per cent moist weight; percentages of organic matter, as per cent dry weight, and percentages of calcium and phosphorus, as per cent inorganic weight. The average difference of duplicate determinations of both calcium and phosphorus amounts to 1 per cent.

Table III shows a summary of the values in Table II. The corrected averages were calculated after discarding all values which differ from the average by more than three times the standard deviation. Some of the percentages of moisture were so low that it was evident that these samples had lost water before their first weighing. These very low values were discarded although they should have been in-

cluded according to standard deviation. On these bases the data were summarized as follows:

	ENAMEL		DENTIN	
	Range	Average	Range	Average
	(per cent)	(per cent)	(per cent)	(per cent)
Moisture	.5– 6.6	*2.8*	4.2–16.7	*11.1*
Organic matter	.7–12.1	*4.9*	5.1–42.0	*24.0*
Ca	31.2–41.1	*36.0*	20.2–46.2	*33.3*
P	14.5–21.4	*17.8*	10.6–21.9	*16.5*

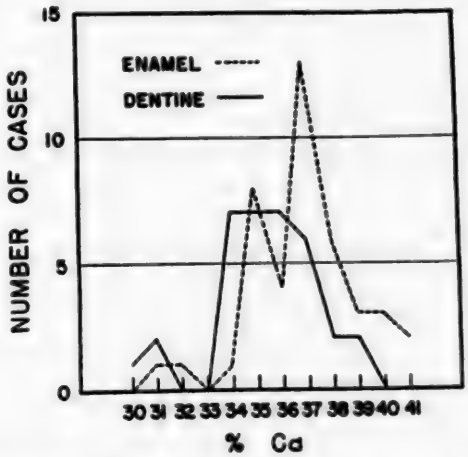


FIG. 1. The distribution of calcium values for deciduous enamel and dentin. Note that the curve for enamel values is to the right of the curve for dentin values, i.e., toward higher calcium percentage.

DISCUSSION

Figs. 1 and *2* summarize the values for the calcium and phosphorus determinations given in Table II. Those which varied considerably from the central tendency were omitted although in some instances the omission was not warranted on the basis of the standard deviation. Only those values for calcium falling between 30 and 41 per cent and only those values for phosphorus falling between 13 and 21 per cent were used. The curves for these two elements follow the normal dis-

tribution curve fairly well. In the case of phosphorus the mode is very pronounced in both enamel and dentin. Although the mode is less pronounced for calcium, the central tendency is still definite. Note that the curve shifts to the right for enamel in both the calcium and phosphorus graphs. It is evident that the calcium and phosphorus graphs follow similar curves.

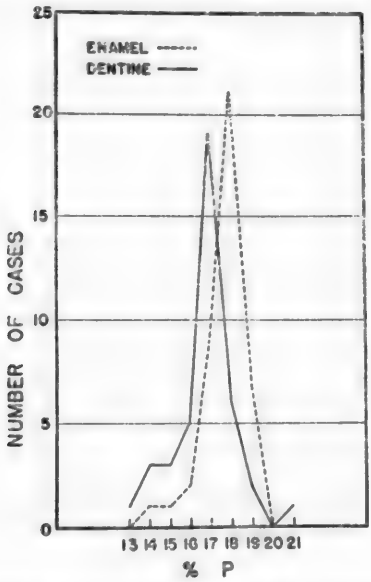


FIG. 2. The distribution of phosphorus values for deciduous enamel and dentin. Note that the curve for enamel values is to the right of the curve for dentin values, i.e., toward higher phosphorus percentages (compare *fig. 1*).

The comparisons of these data with those obtained in earlier analyses are shown in Table IV. In some cases it is not known whether the basis of the percentages of calcium and phosphorus is moist weight, dry weight, or inorganic weight. Sometimes it is not known whether the percentage of organic matter is calculated on the basis of moist or dry weight. No matter what the basis of calculation may be, the calcium:phosphorus per cent ratio is a constant means of comparison. Where these were not given by the original authors, they have been calculated. For deciduous teeth Pepys (1) found a

Ca/P of 2.13; Gassmann (2), 2.23; Murray (3), 2.07; LeFevre and Hodge (4), 2.09; for fetal teeth, Hess, Lewis and Roman (13), 2.05; LeFevre, Bale and Hodge (5), 2.04. These values compare rather well with our present values of 2.06 for enamel and 2.08 for dentin (lower part of Table IV). The theoretical Ca/P for tricalcium phosphate is 1.94 and for hydroxylapatite is 2.15. All of these ratios fall within this range except Gassmann's high value.

TABLE IV

Comparison of analyses of deciduous teeth

AUTHOR	TISSUE[1]	NO. TEETH[2]	MOISTURE[3]	ORGANIC[3]	Ca[3]	P[3]	Ca/P
Pepys (1804)	w.t.	p.s.		20 M	26.4 M	12.4 M	2.13
Gassmann (1908)	w.t.	1(?)	8.76 M	22.8 D?	29.6 D?	13.3 D?	2.23
Hess, Lewis and Roman (1932)	fetal w.t.	12			11.5 mg.	5.08 mg.	2.05
Murray (1936)	D	p.s.			37.6 I	18.2 I	2.07
LeFevre, Hodge (1937)	w.t.	9	11.1 M		34.9 I	16.7 I	2.09
LeFevre, Bale, Hodge (1937)	fetal w.t.	56	6.82 M		34.5 I	16.9 I	2.04
Data from Table III	E	45	2.8 M	4.9 D	36.0 I	17.8 I	2.06
	D	45	11.1 M	24.0 D	33.3 I	16.5 I	2.08

[1] w.t. whole tooth; E. enamel; D. dentin.

[2] p.s. pooled sample.

[3] M—% moist weight; D—% dry weight; I—% inorganic weight.

The values obtained in the present chemical analyses for moisture, organic matter, calcium and phosphorus are classified in Table V, parts A, B, C and D, according to caries class, tooth location, and age and sex of subject, respectively.

There is no significant variation of tooth composition with caries class (Table V, part A).

It is questionable whether the location of a tooth is related to its chemical composition. In Table V, part B, the first and second incisors have much lower values for calcium and phosphorus in the enamel than do the canines and molars. In the dentin, the first incisors have low calcium and phosphorus content, but the second incisors have as much as the other teeth. Due to the small number of

TABLE V[1]

Classification of analytical data

CARIES CLASS	MOISTURE (% MOIST WT.)		ORGANIC (% DRY WT.)		CALCIUM (% INORG. WT.)		PHOSPHORUS (% INORG. WT.)	
	E	D	E	D	E	D	E	D
A. Classification according to caries class								
0	3.8	11.1	5.9	16.9	30.1	30.2	13.9	14.6
	(2)[2]	(2)	(2)	(2)	(2)	(2)	(2)	(2)
I	1.7	12.5	14.9	24.2	36.8	33.0	17.8	15.9
	(18)	(19)	(19)	(18)	(19)	(18)	(19)	(18)
II	2.4	8.0	10.1	25.4	36.2	34.1	17.4	16.6
	(19)	(19)	(19)	(19)	(19)	(19)	(18)	(19)
III	2.9	12.2	14.0	28.0	36.1	31.7	18.8	15.4
	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
B. Classification according to tooth location								
LOCATION								
I	0.9	9.7	37.3	32.6	28.2	26.1	13.5	12.5
	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
II	3.8	11.1	5.4	16.9	30.1	30.2	13.9	14.6
	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
III	5.4	5.8	17.5	18.4	36.2	29.7	17.6	16.7
	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
IV	2.0	9.6	13.6	26.8	37.2	32.1	18.2	15.4
	(18)	(18)	(18)	(18)	(18)	(18)	(17)	(18)
V	2.0	12.0	6.2	24.0	36.4	35.7	17.8	17.2
	(19)	(20)	(20)	(19)	(20)	(19)	(20)	(19)
C. Classification according to age of child								
AGE								
5	2.0	7.8	5.5	23.9	36.9	31.8	18.3	15.7
	(7)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
6	2.5	11.6	11.2	19.8	35.7	32.7	17.2	15.8
	(9)	(9)	(9)	(8)	(9)	(8)	(9)	(8)
7	1.5	7.5	15.8	23.1	33.8	33.8	16.2	15.9
	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
8	1.6	10.2	17.3	30.0	35.3	33.9	17.7	16.4
	(10)	(10)	(10)	(10)	(10)	(10)	(9)	(10)
9	1.8	10.9	2.8	25.6	38.6	36.0	18.3	17.0
	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
10	3.3	13.5	5.7	25.3	38.6	33.3	18.0	16.2
	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
11	4.5	18.6	18.4	21.9	36.7	31.0	17.8	16.9
	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
12	1.7	11.1	7.4	27.5	36.2	32.8	17.6	15.4
	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
D. Classification according to sex of child								
SEX								
Male	2.1	10.9	9.2	25.7	36.1	33.7	17.5	16.2
	(30)	(31)	(31)	(31)	(31)	(31)	(30)	(31)
Female	2.6	9.6	15.8	22.7	36.0	31.7	17.8	15.9
	(14)	(14)	(14)	(13)	(14)	(13)	(14)	(13)

[1] In these tables all values are included; i.e., those greater than three times the standard deviation in Tables II and III are not discarded.

[2] Numbers in parentheses are number of cases.

incisors analyzed, the low averages may be coincidental rather than significant. From Table II, it may be seen that the individual calcium and phosphorus values for incisors are lower than for molars, but these percentages may be aberrant rather than actual.

A peculiar trend may be seen in the values at different ages (Table V, part C). For the years 5, 6 and 7, phosphorus and calcium values decrease in the enamel. During the same years, phosphorus and calcium percentages increase in dentin. The apparent variations with age may be explained in the same way as those for location; i.e., more teeth should be analyzed before any generalization is made as to relations between composition and either age or tooth location.

No significant variations are seen when the values for teeth from males are compared with those from females (Table V, part D).

There are no regular variations for percentages of either moisture or organic matter that would indicate any definite trend.

It has been suggested that these classifications of the teeth are not significant. The caries class, location, age, and sex should have been intercorrelated to make these data more valuable. However, the limited number of tooth samples available made this impossible.

The chemical determinations of deciduous teeth are compared with those of adult teeth in Table VI. The average percentages for permanent tissues were taken from the following authors: of moisture and organic matter—LeFevre and Manly (6), of calcium, phosphorus and calcium:phosphorus ratio in enamel—Armstrong and Brekhus (7), and in dentin—French, Welch, Simmons, LeFevre and Hodge (8).

The average percentage of moisture in deciduous enamel is slightly higher than in adult enamel, but deciduous dentin has a slightly lower moisture content than adult dentin. In neither case is the difference greater than the range of values included by the standard deviations; therefore the differences are not statistically significant. Deciduous enamel and dentin have higher average organic contents than the permanent tissues. In this case also, the differences are not statistically significant. If the moisture and organic contents are summed, the inorganic contents can be calculated by difference. On this basis deciduous enamel has 92.5 per cent; permanent enamel 96.0 per cent; deciduous dentin 67.2 per cent; permanent dentin 69.3 per cent inorganic matter. In each case the permanent tissue appears to be

slightly more calcified than the deciduous. The deciduous enamel samples may have had more enamel cuticle than the permanent. The slightly lower calcification of the deciduous tissues is, however, in line with the greater susceptibility of young enamel to impregnation by stains (14), with the lower density (15, 16) and with the differences in birefringence (17).

The percentage of calcium in enamel is lower for deciduous than permanent teeth. The other calcium and phosphorus values for both enamel and dentin are nearly the same. Ca/P is slightly lower for deciduous than adult teeth.

The calcium:phosphorus per cent ratio is a remarkably constant value (Tables II, III, IV, VI). Its constancy indicates that the tissues

TABLE VI

Comparison of deciduous and permanent teeth

	MOISTURE (% MOIST WT.)		ORGANIC (% MOIST WT.)		Ca (% DRY WT.)		P (% DRY WT.)		Ca/P	
	E	D	E	D	E	D	E	D	E	D
Permanent	2.3	13.2	1.7	17.5	36.1	26.1	17.3	12.6	2.07	2.09
Deciduous	2.8	11.1	4.7	21.7	34.3	26.1	17.0	12.9	2.06	2.08

contain the same fundamental inorganic compound. From the Ca/P ratios calculated, the compound is more like hydroxylapatite than tricalcium phosphate. Through x-ray diffraction study, LeFevre, Bale and Hodge (6) showed that tooth substance (fetal, deciduous, and permanent) is similar to fluorapatite. The fact that the deciduous Ca/P ratio lies between those of fetal and adult teeth (Tables IV, VI) suggests either that with increase in age the fundamental inorganic compound is modified, or that substances are added (perhaps carbonates) which involve an increase in the calcium content relative to the amount of phosphorus present. The principal inorganic molecule of both deciduous and permanent teeth is probably hydroxylapatite. The constancy of composition of these calcified tissues is the outstanding finding of the analyses. The chemical differences between deciduous and permanent enamel and dentin are much less striking than the near identity of their composition (Table VI).

SUMMARY

1. The composition of deciduous teeth is very similar to that of adult teeth.

2. The calcium/phosphorus ratio of the inorganic part of deciduous teeth falls between those for fetal and adult teeth, indicating that the principal inorganic molecule is probably the same (hydroxylapatite) in each.

3. Calcium/phosphorus ratios are 2.06 for deciduous enamel and 2.08 for deciduous dentin.

4. Normal values for deciduous teeth are as follows: *moisture*, enamel 2.8 per cent, dentin 11.1 per cent; *organic matter*, enamel 4.9 per cent, dentin 24.0 per cent; *calcium*, enamel 36.0 per cent, dentin 33.3 per cent; *phosphorus*, enamel 17.8 per cent, dentin 16.5 per cent.

5. Deciduous enamel and dentin composition does not vary significantly when teeth are classified according to caries class, tooth location, age and sex of subject.

REFERENCES TO LITERATURE

(1) Pepys, W. H., quoted by Fox, J., *The Natural History of Human Teeth*, (London, T. Cox and Son: 1803).
(2) Gassmann, T., *Z. physiol. Chem.*, **55**: 455, 1908.
(3) Murray, M. M., *Biochem. J.*, **30**: 1567, 1936.
(4) LeFevre, M. L., and Hodge, H. C., *J. D. Res.*, **16**: 279, 1937.
(5) LeFevre, M. L., Bale, W. F., and Hodge, H. C., *J. D. Res.*, **16**: 85, 1937.
(6) LeFevre, M. L., Manly, R. S., *J. A. D. A.* and *D. Cosmos*, **25**: 233, 1938.
(7) Armstrong, W. D., and Brekhus, P. J., *J. Biol. Chem.*, **120**: 677, 1937.
(8) French, E. L., Welch, E. A., Simmons, E. J., LeFevre, M. L., and Hodge, H. C. *J. D. Res.*, **17**: 401, 1938.
(9) Manly, R. S., and Hodge, H. C., *J. D. Res.*, **18**: 133, 1939.
(10) Crowell, C. D., Hodge, H. C., and Line, W. R., *J. D. Res.*, **14**: 251, 1934.
(11) Sobel, A. E., and Sklersky, S., *J. Biol. Chem.*, **122**: 665, 1938.
(12) Holtz, F., *Biochem. Z.*, **210**: 252, 1929.
(13) Hess, A. F., Lewis, J. M., Roman, B., *D. Cosmos*, **74**: 1053, 1932.
(14) Skillen, W. G., *J. A. D. A.*, **11**: 402, 1924.
(15) Boyd, J. D., Drain, C. L., and Deakins, M. L., *J. D. Res.*, **17**: 465, 1938.
(16) Berghash, S., and Hodge, H. C., unpublished data.
(17) Harders-Steinhauser, M., *Z. für Zellforsch.*, **28**: 274, 1938.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH[1]

Scientific Proceedings of the New York Section
June 6, 1940

Compiled by FRANCES KRASNOW, Ph.D., *Editor*

Abstracts

1. A NEW METHOD FOR DETERMINATION OF THE RELATIONSHIP BETWEEN ORAL INFECTION AND SYSTEMIC DISEASE: VALUE OF BLOOD SEDIMENTATION RATE. *James L. Zemsky*, D.D.S. and *Paul Liebesny*, M.D., Bronx Hospital, New York. The employment of an electrophysical agent to determine possible relationship between systemic involvement and oral infection furnishes a new method for investigation in this field. It is based on the fact that short wave irradiation of either normal tissues or inactive foci has no appreciable effect on blood sedimentation rate, while patients presenting active foci show increased blood sedimentation rate. Although no definite explanation can be offered for this reaction, it is not unlikely that the irradiation with short wave of sufficient intensity causes temporary increase of inflammation and greater permeability for toxins in the region of the focus. In this connection, it should be remembered that certain diseases may revive old inactive foci by altering the level of body immunity which introduces another point, i.e., a provocative factor. The provocation of an oral focus is accomplished through a small special transformer with electrodes adapted for irradiation of individual teeth. Blood specimens are secured prior to, as well as following the irradiation and from these samples the blood sedimentation rate is determined. This new procedure offers a possibility of ascertaining the existence or non-existence of causal relationship between certain foci of infection and systemic disease and may prevent sacrifice of teeth. Case reports complete the presentation.

[1] The cost of publication of these proceedings is paid by the New York Section. Other contributions have not been displaced, these pages being added to those budgeted for Volume 19.

2. Results of culturing root canals by a simple anaerobic method. *Maurice Buchbinder*, D.D.S., School of Dental and Oral Surgery, Columbia University, New York. Although it has long been known that strictly anaerobic bacteria are frequently present in infected root canals, most clinical tests for bacterial growth allow for only aerobic cultivation. A simple method for growing anaerobes suitable for office use is presented. One-half dram screw cap vials, sealed with a thick rubber rim under the cap, are filled with Difco brain-heart-infusion broth containing 0.7 per cent agar. Upon autoclaving the vials the air is driven out, thereby providing anaerobic conditions for several weeks if the cap is tight. Clostridia grew readily in the vials; methylene blue added to the medium was reduced. At the Columbia University root canal clinic, 106 successive pairs of cultures were made in the agar medium indicated above and in one of the same composition without agar—one cotton point being stabbed into the agar, the other dropped into the broth. 34 showed growth in both tubes, 53 were sterile in both, 14 showed growth in the agar but not in the broth and 5 showed growth in the broth but not in the agar. A distinctly higher proportion of positive results were thus obtained by the anaerobic method, indicating the presence of anaerobes in the root canals.

3. Daily observations on the formation of cementum, periodontal membrane and bone with the use of boiled cow bone powder. *Frank E. Beube*, D.D.S., Department of Periodontology, School of Dental and Oral Surgery, Columbia University, New York. It was noted that boiled bone powder inserted into the cavity arising from the removal of the buccal plate, periodontal membrane and cementum over roots, resulted in the generation of new alveolar bone, periodontal membrane and cementum. Up to the sixteenth day bone particles could be observed in the histological sections. They were much more abundant in the earlier days. The new cementum or osteoid tissue was the first structure to be laid down over the denuded dentin after the formation of early connective tissue. This was seen on the fourteenth day. A few days later trabeculation of the alveolar bone with early connective tissue formation between the new cementum and the trabeculating bone began to appear. On the twenty-seventh day, there was increased trabeculation and more

bone was laid down, but no true periodontal membrane as yet had formed; the connective tissue however was losing its early histological appearance. A well formed periodontal membrane was noted on the thirty-fifth day. The cementum showed increasing thickness as the days passed. The manner by which the bone particles were utilized was not determined.

4. REPORT OF A CORRELATION BETWEEN CARIES AND PERIODONTAL DISEASE. *Samuel Charles Miller*, D.D.S. and *Benjamin B. Seidler*, A.B., D.D.S., College of Dentistry, New York University, New York. A series of 1,003 cases were studied radiographically to determine whether a correlation exists between caries and periodontal disease. The cases were selected at random and ranged in age from 11 to 60 years. Bodecker's modified dental caries index was employed in the computation of a particular radiographic series. The amount of alveoloclasia was used as the index of periodontal disease. It was found that females were more resistant to alveoloclasia and were more susceptible to caries; males evidenced the reverse relationship. In order to determine the coefficient of correlation, Pearson's product moment method was used. The coefficient of correlation for the entire group of 1,003 cases was found to be +2. The coefficient, determined by the same method, on a group of cases of from 31 to 45 years of age, was found to be −8. Results are considered to be insignificant if they are less than 35. It seems most likely, therefore, that there is no relation between the two factors. Given a certain caries index, that patient may evidence no alveoloclasia, or show any amount.

Misplacement of the Tribute to Dr. Kronfeld

By accident, in the arrangement of the parts of the Proceedings of the meeting of the International Association for Dental Research in Philadelphia, on March 13 and 14, the tribute to Dr. Kronfeld, now on pages 266–276, does not precede the presidential address, which at the meeting succeeded the tribute and was in turn followed by the inaugural address. The intended sequence of these three parts of the Proceedings is indicated on page 312.